Immunohistochemistry in the diagnosis of cutaneous infections

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Thesis for the fulfillment of the
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Department of Internal Medicine

TESIS DOCTORAL

Immunohistochemistry in the Diagnosis of Cutaneous Viral and Bacterial Infections

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CERTIFICA:

Que **Dña. Ana María Molina Ruiz** ha realizado bajo mi dirección el trabajo **"Immunohistochemistry in the Diagnosis of Cutaneous Viral and Bacterial Infections"** que a mi juicio reúne las condiciones para optar al Grado de Doctor.

Para que así conste, firmo el presente certificado en Madrid a 3 de septiembre del año dos mil catorce.

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May 28, 2014

To Whom it May Concern

Gentlemen,

I have read Dr. Ana María Molina Ruiz Doctoral Thesis entitled "**Immunohistochemistry in the diagnosis of cutaneous viral and bacterial infections"** and I think that this research study on immunohistochemistry is very interesting both for dermatologists and pathologists nowadays because several of the antibodies reviewed in this paper are relatively new and their real applicability on daily routine has not been properly established yet.

IHC continues to be one of the main adjunctive methods to conventionally stained sections in histopathology. This is mainly related to the fact that it is a relatively simple, fast, and inexpensive method. In this review, the authors highlight the main immunohistochemical techniques that have been used and continue to evolve in the diagnosis of mucocutaneous viral and bacterial infections, and discuss their applicability.

Furthermore, I don't think such an exhaustive study of the immunohistochemistry of cutaneous infections has been published previously, and therefore this work will probably be used frequently as an expert consult paper, both by dermatologists and dermatopathologists.

Sincerely yours,

Priv.-Doz. Dr. med. Heinz Kutzner

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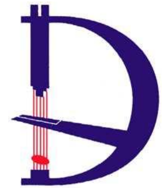

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To whom it may concern

I have read with great interest the Doctoral Thesis by **Dr. Ana María Molina Ruiz**, titled "**Immunohistochemistry in the diagnosis of cutaneous viral and bacterial infections**".

In my opinion this research study on immunohistochemistry of infectious diseases is crucial for both dermatologists and dermatopathologists, particularly because several of the antibodies discussed and illustrated in the study are new and little-known in the dermatologic and dermatopathologic communities. In addition, as infectious disorders are a major health problem, and as a precise diagnosis is a pre-requisite for the correct treatment, this study addresses a very important problem and offers novel ways of properly managing these patients.

The use of a continuously increasing number of newly raised and commercially available antibodies for immunohistochemistry has tremendously broadened the applicability of this technique in routine diagnostic procedures, and this is also relevant for the diagnosis of different types of infections. Immunohistochemistry represents a sensitive, specific and relatively unexpensive technique that allows a precise characterization of viral and bacterial infections in biopsy specimens, which may be very difficult or even impossible to diagnose using routine microscopy alone.

Furthermore, to the best of my knowledge this is the only exhaustive and complete study of the immunohistochemistry of cutaneous infections that has been published in the medical literature, thus representing a crucial reference and source of relevant information for both dermatologists and dermatopathologists.

Sincerely,

Prof. Dr. Lorenzo Cerroni

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To my family Eustoquio, Eladia, Rosa and Javier.

“Science is a way of thinking much more than it is a body of knowledge"

Carl Sagan

Aknowledgments

Quiero expresar mi más sincero agradecimiento al Profesor **Luis Requena.** Él es el científico más brillante que conozco y el motor de un servicio de Dermatología al que hace brillar junto él. Sin su infinita ayuda, apoyo, confianza, paciencia y entusiasmo hubiera sido imposible la realización de este proyecto. Trabajar al lado de alguien tan excepcional y con un cerebro tan bien estructurado científicamente convierte la dermatopatología en algo emocionante.

This thesis would not have been possible without the help of **Dr. Heinz Kutzner** who generously contributed to the search and purchase of the immunohistochemical stains that were necessary for this project. His extremely helpful ideas, suggestions and quick responses while reviewing the articles were also vital in the process.

I would also like to acknowledge **Dr. Lorenzo Cerroni** for his continuous support with this and other scientific projects. For teaching me Dermatopathology since the very first years of my residency and for coming to Madrid to be present during the presentation of the project.

Gracias a mi padre, **Eustoquio Molina**, por haberme inculcado la ilusión por la ciencia y hacer de esa ilusión un hecho con sus sabios consejos y constante ayuda; a mi madre, **Eladia Ruiz**, por enseñarnos a valorar la importancia de estudiar y aprender cosas nuevas de una forma libre e independiente; y a **Javier**, mi pareja, por soportar mis largas tardes de ausencia frente al ordenador y por hacer que mi vida sea tan divertida y feliz.

A todos ellos muchísimas gracias ayudarme en este proyecto.

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Abbreviations

Abbreviations

IHC: Immunohistochemistry

IHQ: Inmunohistoquímica

PCR: Polymerase chain reaction

ISH: In situ hybridization

ELISA: Enzyme-linked immunosorbent assay

HSV-1: Herpes simplex virus-1

HSV-2: Herpes simplex virus-2

VZV: Varicella zoster virus

CMV: Cytomegalovirus

EBV: Epstein-Barr virus

HHV-6: Human herpesvirus 6

HHV-7: Human herpesvirus 7

HHV-8: Human herpesvirus 8

LMP-1: Latent membrane protein 1

EBNA-2: Epstein-Barr nuclear antigen 2

EBERs: Epstein-Barr-encoded RNAs

HHV-6A: Human Herepesvirus-6 variant A

HHV-6B: Human Herepesvirus-6 variant B

KS: Kaposi´s sarcoma

KSHV: Kaposi´s sarcoma-associated herpesvirus

LNA-1: Latent nuclear antigen-1

HIV: Human immunodeficiency virus

HPV: Human papillomavirus

EV: Epidermodysplasia verruciformis

SCC: Squamous intraepithelial lesions

H-E: Hematoxylin and eosin

PVB19: Parvovirus B19

PPGSS: Purpuric "gloves-and-socks" syndrome

VP: Viral protein

HPyV: Human polyomavirus

MCPyV: Merkel cell polyomavirus

TSPyV: Trichodysplasia spinulosa polyomavirus

MCC: Merkel cell carcinoma

DIPS: Detection of integrated polyomavirus sequences

TS: Trichodysplasia spinulosa

WHO: World Health Organization

SLAM: signalling lymphocyte activation molecule

HFMD: Hand-foot-and-mouth disease

HTLV-1: Human T-cell lymphotropic virus type I

IL: Interleukin

ATLL: Adult T-cell leukemia/lymphoma

HBZ: Basic leucine zipper

EHF: Ebola hemorrhagic fever

FFPE: Formalin-fixed and paraffin-embedded

GAS: Group A Streptococcus

CDC: Centers for Disease Control

IDU: Injecting drug users

PAS: Periodic acid-Schiff

MTBC: Mycobacterium tuberculosis complex

TB: Tuberculosis

LAM: Lipoarabinomannan

HAART: Highly active antiretroviral therapy

WS: Warthin-Starry

AARB: Acid alcohol resistant bacilli

FFM: Focus floating microscopy

LGV: lymphogranuloma venereum

MSM: men who have sex with men

MOMP: major outer membrane protein

DFA: direct fluorescent antibody

MALT: mucosa associated lymphoid tissue

RMSF: Rocky Mountain spotted fever

SFG: Spotted fever group

IFA: Immunofluorescent antibody assay

WD: Whipple disease

SCC: Squamous cell carcinoma

Introduction

Introduction

Immunohistochemistry

Immunohistochemistry (IHC) is an indispensable tool for the surgical pathologist that is constantly evolving and expanding. It has wide applications in everyday practice, such as determination of the nature, lineage, or differentiation of normal cells or tumors; distinguishing between benign and malignant neoplasms; assessment of the likely primary site for tumors of uncertain origin; and demonstration of the existence of microorganisms.[1] With the availability of highly effective antigen retrieval techniques, highly sensitive immunohistochemical detection systems, and a wide spectrum of new antibodies and with better understanding of the molecular alterations in tumors, IHC is also playing an increasingly important role for dermatopathologists.[2] New immunohistochemical targets are continually being found, contributing to more accurate diagnosis and classification of cutaneous disorders.

In selected circumstances, IHC can also complement or even replace molecular analysis, by serving as a screening tool to triage samples for the latter analysis. Compared with molecular studies, immunohistochemical studies are less expensive and less labor-intensive, can be performed in routine diagnostic laboratories, and can usually be completed in a much shorter time. Therefore, the use of widespread immunohistochemical stains in recent years has given the pathologist in general and the dermatopathologist specifically many new weapons in their diagnostic armamentarium.[3]

IHC covers a group of immunostaining techniques in which labeled antibodies are used to detect the presence of antigens in cells or tissues. The principle of IHC lies in the ability of antibodies to bind specifically to their respective antigens. The resulting reaction can only be visualized when the antibody is labeled with a substance that absorbs or emits light or produces color.[4] The principle of IHC has been known since the 1930s, but it was not until 1942 that the first IHC study was reported. Coons et al. used fluorescent-labeled antibodies to identify *Pneumococcal* antigens in infected tissue. Since then, improvements have been made in protein conjugation, tissue fixation methods, detection labels and microscopy, making IHC a routine and essential tool in diagnostic and research laboratories.[5]

Immunofluorescence techniques rely on the use of fluorescein-labeled markers, which, when exposed to ultraviolet light, emit visible light of varying wavelengths depending on the nature of the compound used. Direct immunofluorescence is used widely in the diagnosis of skin diseases, a field in which it has very specific indications. In particular it is used to diagnose bullous diseases, vasculitis, and certain types of tumors. Although it is more sensitive than immunostaining, immunofluorescence has certain disadvantages, including loss of fluorescence over time, the need for a specialized light microscope, and poor visualization of morphologic features. Furthermore, the resulting reactions need to be photographed each time for documentation purposes.[6]

Immunoperoxidase techniques involve the use of enzyme labels that convert a colorless substrate into a colored one. The most widely used enzymes are peroxidase and alkaline phosphatase, and the most widely used substrates are diaminobenzidine,

amino ethylcarbazole, and nitroblue tetrazolium, which, respectively, produce a brown, red, and blue color. These markers can be attached, or conjugated, directly to the primary antibody, or indirectly using secondary antibodies or substances such as biotin and protein A.[6]

Immunohistochemistry in the Diagnosis of Cutaneous Infections

The identification of pathogens is of vital importance for the adequate treatment of infections, however significant difficulties continue to exist in the diagnosis of many infectious diseases. With an increasing number of skin specimens from patients with disorders suspected of being infectious, identification of infectious agents has become a time-consuming task in routine dermatopathology, where there is growing demand for a simple, fast, cost-effective, and highly sensitive screening tool for the detection of microorganisms.

Traditionally, microbial identification in infectious diseases has been made primarily by using conventional microscopy, tissue cultures and serologic assays. Some microorganisms have distinctive morphologic characteristics that allow their identification in formalin-fixed tissues using routine and special stains. Nevertheless, in several instances it is difficult or even impossible to identify an infectious agent specifically by conventional morphologic methods. In addition, the sensitivity of tissue cultures is limited, particularly in infections with low pathogen numbers or the inability to grow the pathogen in culture. In addition, fresh tissue is not always available for culture, and culture alone cannot distinguish colonization from tissue invasion. Also,

serologic results can be difficult to interpret in the setting of immunosuppression or when only a single sample is available for evaluation.

Many histochemical "special" stains and a growing number of specific monoclonal antibodies, as well as advanced molecular methods, provide a huge armamentarium for the detection of microorganisms.[2] The molecular diagnosis of infectious disease has been growing considerably over the past decade and has emerged as an useful tool in the diagnosis of cutaneous infections that are challenging and ambiguous on conventional histopathology, thus improving our diagnostic accuracy.[1] Nucleic acid amplification techniques, such as polymerase chain reaction (PCR), ligase chain reaction, transcription-mediated amplification, and nucleic acid sequence-based amplification, provide highly accurate diagnosis of numerous bacterial, viral, fungal, and parasitic infections involved in a variety of dermatologic diseases. In addition, signal amplification with hybrid capture, branched-DNA assays, and in situ hybridization (ISH) have been used to detect numerous viral pathogens with high degrees of sensitivity and specificity. New technology that involves the use of DNA and protein microarrays has also enabled the detection of a variety of genes and gene mutations.[7-9]

IHC has a definite role to play in the diagnosis of cutaneous infections. However, applications of IHC in this difficult area require a certain degree of sophistication and also considerable experience. Although several antibodies have been developed to aid in the immunohistochemical diagnosis of many infectious diseases, only a small subset of these antibodies is useful in dermatopathology.[10] Moreover, few studies have reviewed IHC utilization by dermatopathologists in the

setting of cutaneous infections by virus and bacteria, especially for the more recently developed stains. Therefore in this study we describe the immunohistochemical findings in the histopathological diagnosis of cutaneous viral and bacterial infections, as well as the potential pitfalls commonly encountered in practice. Emphasis is placed on new stains as well as novel uses of existing stains. As always, immunohistochemical data has been interpreted in the light of clinical and routine histologic findings.

Introducción

Inmunohistoquímica

La inmunohistoquímica (IHQ) es una herramienta de trabajo indispensable para el patólogo general, y un campo en continuo crecimiento y expansión. Es un arma diagnóstica que presenta varios campos de aplicación en la práctica diaria como son: la determinación de la naturaleza, estirpe y diferenciación de las células tumorales; la distinción entre neoplasias benignas y malignas; la estimación de la procedencia de tumores de origen desconocido; y la demostración de la presencia de microorganismos en muestras de tejido[1]. Con la incorporación de técnicas de recuperación antigénica altamente efectivas, sistemas de detección IHQ extremadamente sensibles, un amplio espectro de nuevos anticuerpos, y una mejor comprensión de las alteraciones moleculares de las lesiones tumorales, la IHQ juega también un papel cada vez más importante para los dermatopatólogos[2]. Cada día se descubren nuevas dianas IHQs que contribuyen a un diagnóstico más preciso y mejor clasificación de las enfermedades cutánenas.

En algunas circunstancias especiales, la IHQ puede también complementar o incluso sustituir las técnicas moleculares, y servir como una técnica de "screening" que permita seleccionar las muestras que precisarán estudios moleculares posteriores. Al comparar la IHQ con las técnicas de diagnóstico molecular, la IHQ resulta una técnica más rápida, menos costosa económicamente, requiere menos trabajo de laboratorio, y puede realizarse en laboratorios no especializados. Todo ello ha motivado el creciente

uso de tinciones IHQs en los últimos años, confiriendo a los patólogos en general y más concretamente a los dermatopatólogos nuevas armas diagnósticas que complementen a las ya existentes[3].

La IHQ comprende un grupo de técnicas de inmunotinción que permiten demostrar una variedad de antígenos presentes en las células o los tejidos utilizando anticuerpos marcados. Estas técnicas se basan en la capacidad de los anticuerpos para unirse específicamente a los correspondientes antígenos y la reacción se hace visible sólo si el anticuerpo está marcado con una sustancia que absorbe o emite luz o produce coloración[4]. Las bases de la IHQ se conocen desde principios de 1930, pero hasta 1942 no se publicó el primer estudio con IHQ. Coons y colaboradores utilizaron anticuerpos marcados con fluoresceína para identificar antígenos de *Pnemococo* en muestras de tejido lesional. Desde entonces, se han llevado a cabo múltiples mejoras en la conjugación de proteinas, técnicas de fijación de tejidos, idenficación de antígenos y microscopía que han permitido situar a la IHQ como una técnica esencial y de rutina en laboratorios generales y de investigación en todo el mundo[5].

En las técnicas de inmunofluorescencia se utilizan como marcadores compuestos de fluoresceína que bajo luz ultravioleta emiten luz de longitud de onda visible, que depende de la naturaleza del compuesto. La inmunofluorescencia directa se utiliza frecuentemente en el diagnóstico de las enfermedades cutáneas en donde tiene indicaciones muy precisas, como las enfermedades ampollares, las vasculitis o el diagnóstico de determinadas neoplasias. Pese a ser más sensible que la IHQ, la inmunofluorescencia presenta algunos inconvenientes, como son la pérdida de la fluorescencia con el tiempo, la necesidad de una microscopía con luz especializada y la

pobreza del detalle morfológico. Además, para documentar cada caso, es necesario fotografiar la reacción[6].

En las técnicas de inmunoperoxidasa se utilizan como marcadores enzimas capaces de hacer cambiar de color un sustrato incoloro. Por ejemplo, las enzimas más frecuentemente utilizadas son la peroxidasa y la fosfatasa alcalina y los sustratos más comunes son la diaminobenzidina (color marrón), el aminoetilcarbazol (color rojo) y el nitroazul de tetrazolio (color azul). Estos marcadores pueden unirse (conjugarse) directamente al anticuerpo primario o bien indirectamente mediante otros anticuerpos (secundarios) o sustancias como biotina o proteína A[6].

Inmunohistoquímica en el Diagnóstico de las Infecciones Cutáneas

La correcta identificación de los microorganismos resulta esencial para el tratamiento adecuado de las infecciones, sin embargo, hoy en día todavía siguen existiendo importantes dificultades en el diágnostico de muchas enfermedades infecciosas. El número de muestras cutáneas procedentes de pacientes con sospecha de padecer una enfermedad infecciosa es cada vez mayor, y la correcta idenfiticación de los organismos implicados en la producción de estas enfermedades se ha convertido en una tarea laboriosa en dermatopatología, donde cada vez existe una mayor demanda por encontrar una técnica de "screening" que resulte a la vez simple, rápida, coste-efectiva y altamente sensible en la detección de microorganismos.

Tradicionalmente, la identificación de los microorganismos implicados en las infecciones cutáneas se ha realizado mediante microscopía óptica, cultivos de muestras cutáneas y estudios serológicos. Algunos microorganismos presentan

características morfológicas específicas que permiten su identificación en muestras de tejido fijado en parafina mediante el uso de tinciones de rutina o especiales. Sin embargo, en múltiples ocasiones, la identificación de estos microorganismos resulta muy difícil o incluso imposible con el uso aislado de los métodos morfológicos convencionales. Además, la sensibilidad de los cultivos es limitada, especialmente en infecciones con escaso número de microorganismos o en casos en los que el patógeno responsable no crece en los cultivos habituales. Además, no en todos los casos existen muestras de tejido disponibles para cultivo, y los cultivos per se no distinguen si un patógneo es un mero colonizante o el agente causal de la enfermedad. Por último, la interpretación de los estudios serológicos pueden resultar complicada en el contexto de pacientes inmunosuprimidos, o cuando sólo se dispone de una única muestra para el análisis.

En este sentido, las tinciones histoquímicas "especiales", el amplio número de anticuerpos monoclonales y métodos moleculares en constante desarrollo, constituyen un importante armamento en la correcta identificación de los microorganismos.[2] El diagnóstico molecular de las enfermedades infecciosas ha experimentado un importante crecimiento en la última década y hoy en día constituye una herramienta muy valiosa en el diagnóstico de enfermedades infecciosas cutáneas que son difíciles de diagnosticar con el uso aislado de la histopatología convecional.[1] Las técnicas de amplificación de ácidos nucléicos, como la reacción en cadena de la polimerasa (PCR), la reacción en cadena de la ligasa, la amplificación mediada por transcripción, y la amplificación de ácidos nucleicos basada en la secuencia, pueden diagnosticar de forma muy precisa un número importante de enfermedades cutáneas

debidas a la infección por virus, bacterias, hongos y parásitos. Además, la amplificación de la señal con captura de híbridos, los ensayos de ADN ramificado, y la hibridación in situ (HIS) también han sido empleados en la detección de numerosos patógenos virales con un alto grado de sensibilidad y especificidad. Por último, las nuevas tecnologías que implican el uso de ADN y microarrays de proteínas han permitido la detección de una amplia variedad de genes y mutaciones genéticas.[7-9]

En resumen, la IHQ juega un papel definitivo en el diagnóstico de las enfermedades infecciosas cutáneas. Sin embargo, el uso adecuado de la IHQ en este campo requiere un grado considerable de experiencia, ya que aunque el número de anticuerpos que se han desarrollado para el diagnóstico las enfermedades infecciosas es muy extenso, solo algunos de estos anticuerpos son realmente útiles en dermatopatología.[10] Además, existen pocos estudios que analicen la utilidad de la IHQ en el contexto de las infecciones cutáneas por virus y bacterias, especialmente en el caso de las tinciones IHQs de reciente desarrollo. Por todo ello, este estudio describe los hallazgos inmunohistoquímicos en el diagnóstico histopatológico de las infecciones cutáneas por virus y bacterias, con hincapié en las nuevas tinciones IHQs así como en los nuevos usos de tinciones IHQs ya existentes. Como siempre, los resultados obtenidos han sido interpretados en el contexto de los hallazgos clínicos e histopatológicos de cada caso, y teniendo en cuenta las posibles dificultades asociadas a la práctica diaria.

Introduction references

1. Chan JK, Ip YT, Cheuk W. The utility of immunohistochemistry for providing genetic information on tumors. Int J Surg Pathol. 2013;21:455-475.

2. Demicco EG. Sarcoma diagnosis in the age of molecular pathology. Adv Anat Pathol. 2013;20:264-274.

3. Kutzner H, Argenyi ZB, Requena L, Rütten A, Hügel H. A new application of BCG antibody for rapid screening of various tissue microorganisms. J Am Acad Dermatol. 1998;38:56-60.

4. Abbas O, Bhawan J. Infections in dermatopathology: emerging frontiers. Am J Dermatopathol. 2012;34:789-796.

5. Coons AA, et al. J Immunol. 1943;45, 159-170.

6. Fuertes L, Santonja C, Kutzner H, Requena L. Immunohistochemistry in dermatopathology: a review of the most commonly used antibodies (part I). Actas Dermosifiliogr. 2013;104:99.

7. Sra KK, Torres G, Rady P, Hughes TK, Payne DA, Tyring SK. Molecular diagnosis of infectious diseases in dermatology. J Am Acad Dermatol. 2005;53:749-765.

8. Dadzie OE, Neat M, Emley A, Bhawan J, Mahalingam M. Molecular diagnostics-an emerging frontier in dermatopathology. Am J Dermatopathol. 2011;33:1-13.

9. Sra KK, Babb-Tarbox M, Aboutalebi S, Rady P, Shipley GL, Dao DD, Tyring SK. Molecular diagnosis of cutaneous diseases. Arch Dermatol. 2005;141:225–241.

10. Wasserman J, Maddox J, Racz M, Petronic-Rosic V. Update on immunohistochemical methods relevant to dermatopathology. Arch Pathol Lab Med. 2009;133:1053–106.

Research Objectives

Research objectives

Main Objectives

1. The main goal of this study is to describe the immunohistochemical findings obtained using specific commercially available antibodies to a wide range of viral and bacterial pathogens involved in cutaneous infections.

2. Another important objective of this study is to test newer stains or new applications of older stains, with the purpose of providing novel information on its utilization in cutaneous viral and bacterial infections, and describing the diagnostic utility of IHC in positive cases.

Secondary Objectives

1. For the cutaneous infections tested in this study, the objective is to describe the following immunohistochemical information:

a) The cutaneous structures where viral and bacterial pathogens are expressed in the course of infection.

b) The specific cells in which viral and bacterial pathogens are expressed in positive cases.

c) The pattern of distribution of the viral and bacterial antigens within these cells (nuclear or cytoplasmatic).

2. For the cutaneous infections not tested in this study (because monoclonal antibodies for immunohistochemical detection are not commercially available), the

objective is to provide a thorough review of the current scientific literature on the topic of immunohistochemical detection of viral and bacterial pathogens in the skin. This information is intended to be useful both for Dermatologists and Dermatopathologists.

Material and Methods

Material and methods

This is a descriptive study based on the collection file material of the authors from 1995 to 2014, which included several hundreds of cutaneous tissue samples from patients with a wide range of different viral and bacterial infections with cutaneous involvement. The main goal of the study was to immunohistochemically detect specific viral and bacterial pathogens infecting the skin, and describe the particular immunohistochemical findings of the cases in which a positive detection was achieved.

The total number of biopsy samples in our files and the relative frequency of each one of them were beyond the areas of interest of the study.

Medline search

For each cutaneous viral and bacterial infection, a comprehensive search of the previous scientific literature was performed in Medline. Relevant articles, chapters, and books published until 1 June 2014, regarding the immunohistochemical detection of viral and bacterial pathogens in the course of cutaneous infection were identified. The search was conducted using the key term combinations: "immunohistochemistry", "cutaneous infections", "viral infections", "bacterial infections", and other relevant terms specific for each different infection. Papers written in English or Spanish were included. Publications found through this indexed search were reviewed for further relevant references. We also included case reports that described the

immunohistochemical detection of particular viral or bacterial pathogens in the skin and other organs.

The search strategy covered three facets:

1) Identify which viral and bacterial pathogens had been immunohistochemically detected using specific monoclonal antibodies in human tissues.

2) Identify which of these monoclonal antibodies for viral and bacterial pathogens had been tested in cutaneous tissue samples of patients with infectious diseases.

3) Identify which of these monoclonal antibodies were commercially available.

Tissue samples

Formalin-fixed, paraffin-embedded specimens of infectious disorders were selected from the files of the Pathology Department, Fundación Jiménez Díaz, Madrid, collected from 1995 to 2014. These files contained clinical data including symptoms and biopsy day for each patient. In all cases the diagnosis had been confirmed by histochemical stains, clinical data, or additional laboratory investigations. Histochemical stains (periodic acid–Schiff, Grocott methenamine silver, mucicarmine, Ziehl-Neelsen, Gram) were performed according to established procedures.

Representative sections stained with hematoxylin-eosin were evaluated for histological features by the authors, and blocks were selected on the basis of suitable tissue to perform immunohistochemical studies. In addition, normal tissues (variable with each microorganism, but mostly lymphatic nodes and skin) were placed as internal controls.

This study was deliberately restricted to diseases in which the detection of organisms could be achieved using commercially available antibodies. Not included were microorganisms that have shown positive immunohistochemical detection with private non-commercially available antibodies described in the scientific literature or developed for research purposes at the Centers of Disease Control (CDC) in Atlanta.

Immunohistochemistry

Immunohistochemical staining was performed both at the Pathology Department of Fundación Jiménez Díaz University Hospital in Madrid and the Dermatopathology Laboratory at Friedrichshafen, Germany. For immunohistochemical examination, formalin-fixed tissue sections (4-µm thick) were deparaffinized in xylene and redehydrated in graded alcohols and distilled water. Automated immunostaining was performed on a BioTek Solutions TechMate 500 (Dako, Hamburg, Germany) where 1-hour incubation with a primary antibody was performed.

Immunohistochemical staining was performed using the following 25 commercially available primary antibodies against biologically relevant proteins of different viral and bacterial pathogens:

Antibody Against	Type/Clone
Herpes simplex virus-1 (HSV-1)	Rabbit polyclonal, DAKO
Herpes simplex virus-1 (HSV-2)	Rabbit polyclonal, DAKO
Varizella zoster virus (VZV)	Monoclonal, mouse C90.2.8, Menarini
Epstein-Barr virus (EBV)	Monoclonal, mouse CS1-4, DAKO
Cytomegalovirus (CMV)	Monoclonal, mouse CCH2 + DDG9, DAKO
Human herpesvirus 8 (HHV-8)	Monoclonal, mouse 13B10, Menarini
Papillomaviruses (HPV)	HPV L1: monoclonal, mouse, Cytoimmune diagnostics HPV cocktail: monoclonal, mouse BPV-1/1H8+, CA Zytomed systems
Parvovirus B19 (PVB19)	Monoclonal, mouse R92F6, Menarini
Merkel cell polyomavirus (MCPyV)	Monoclonal, mouse CM2B4, Santa Cruz Biotechnology
SV40 [Trichodysplasia spinulosa polyomavirus (TSPyV)]	Monoclonal, mouse Pab101, BD Biosciences
Human T-cell lymphotropic virus type I (HTLV-1)	Monoclonal, mouse 1A3, Biozol
Measles	Polyclonal, rabbit, Acris
Enterovirus	Monoclonal, mouse, DAKO

Antibody Against	**Type/Clone**
Staphylococcus aureus	Anti-*S. aureus,* KPL BacTrace, polyclonal, goat; Medac
Streptococcus pyogenes (group A)	Anti-*Streptococcus A*, polyclonal, rabbit; Biorbyt
Mycobacterium bovis BCG	Anti-*M. bovis* BCG, polyclonal, rabbit; DAKO
Mycobacterium tuberculosis	Anti-*M. tuberculosis*, polyclonal, rabbit; Zytomed Systems
Treponema pallidum	Anti-*T. pallidum*, polyclonal, rabbit; Zytomed Systems
Borrelia burgdorferi	Anti-*Borrelia*, polyclonal, rabbit; Biogenesis
Borrelia species	Anti-*Borrelia* species, KPL BacTrace, polyclonal, goat; Medac
Chlamydia trachomatis	Anti-*C. trachomatis*, monoclonal (BDI815), mouse; Acris Antibodies
Bartonella henselae	Anti-*B. henselae*, monoclonal (H2A10), mouse; Zytomed Systems
Bartonella quintana	Anti-*B. quintana*; Xceltis
Neisseria meningitidis	Anti-*N. meningitidis*, polyclonal, rabbit; Thermo Fisher Scientific

Results

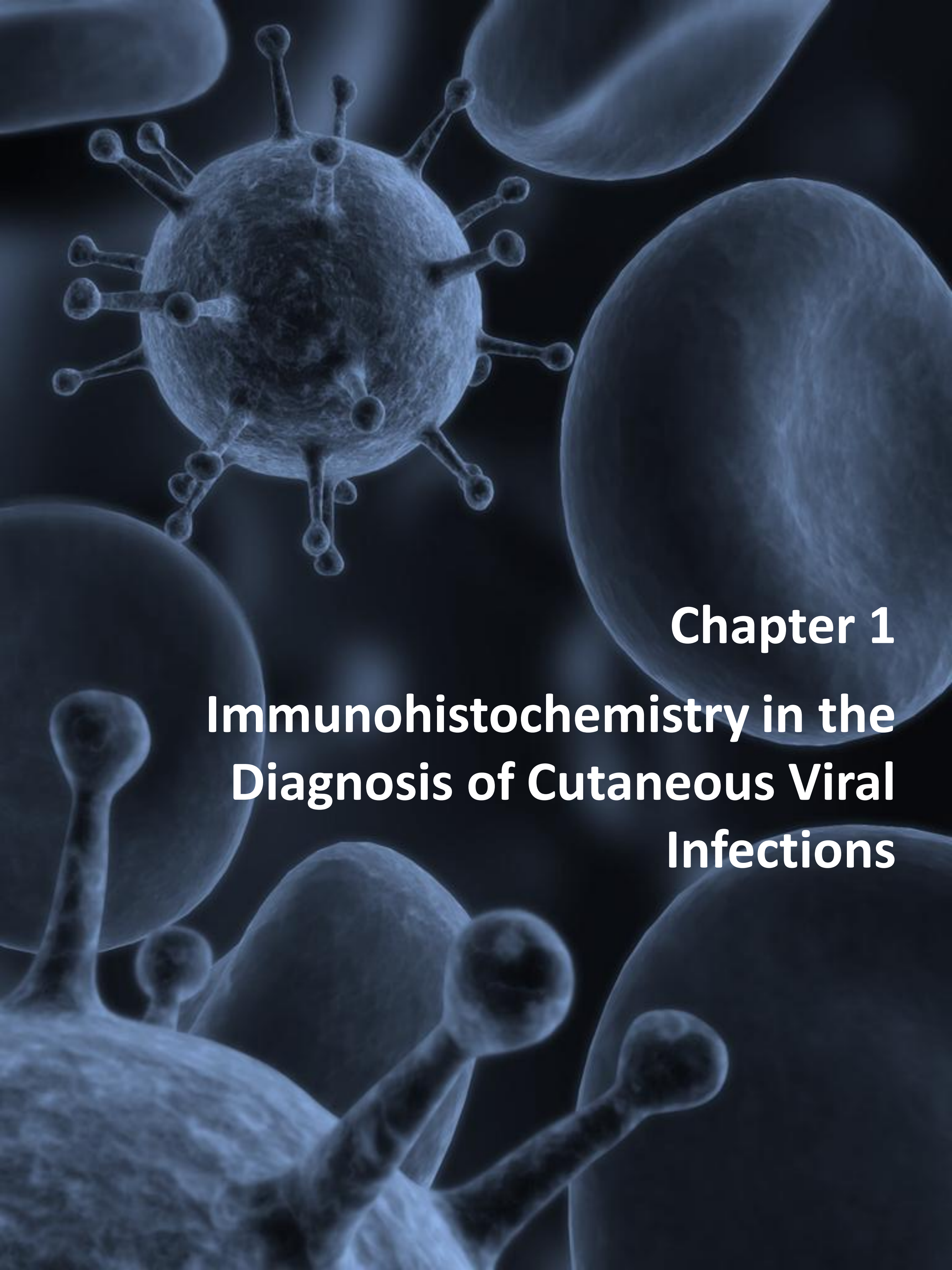
Results

The results of this study have been published as the following critical review articles in the American Journal of Dermatopathology:

1. Molina-Ruiz AM, Santonja C, Rütten A, Cerroni L, Kutzner H, Requena L. Immunohistochemistry of viral infections. Part I. Cutaneous viral infections by herpesviruses and papillomaviruses. American Journal of Dermatopathology. 2014 Aug 28. [Epub ahead of print] PubMed PMID: 25171431.

2. Molina-Ruiz AM, Santonja C, Rütten A, Cerroni L, Kutzner H, Requena L. Immunohistochemistry of viral infections. Part II. Cutaneous Viral Infections by Parvoviruses, Poxviruses, Paramyxoviridae, Picornaviridae, Retroviruses and Filoviruses. American Journal of Dermatopathology. 2014 Aug 28. [Epub ahead of print] PubMed PMID: 25171430.

3. Molina-Ruiz AM, Cerroni L, Kutzner H, Requena L. Immunohistochemistry in the diagnosis of cutaneous bacterial infections. American Journal of Dermatopathology. 2014. (In press).



Chapter 1

Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections

ARTICLE 1

Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections - Part I. Cutaneous Viral Infections by Herpesviruses and Papillomaviruses

Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections—Part I. Cutaneous Viral Infections by Herpesviruses and Papillomaviruses

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Background: Cutaneous viral infections are of increasing clinical importance, particularly in patients who are immunocompromised.

Objective: The use of immunohistochemistry (IHC) in the diagnosis of cutaneous infections provides a rapid morphological diagnosis and can be applied to confirm the diagnosis of specific viral infections that may be difficult to diagnose with certainty using routine microscopy alone, thus facilitating clinical decisions in patient care.

Methods: Several immunostains for specific viruses that have been useful in dermatopathology are reviewed. Emphasis is placed on new stains and novel uses of existing stains.

Results: This article is an up-to-date overview of the potential uses and limitations of IHC in the histopathologic diagnosis of cutaneous viral infections by herpesviruses and papillomaviruses.

Limitations: Whereas specific monoclonal antibodies effectively distinguish infections by herpes simplex virus-1, herpes simplex virus-2, varicella zoster virus, Epstein–Barr virus, and cytomegalovirus, IHC does not distinguish between the 120 antigenically distinct strains of human papillomavirus.

Conclusions: IHC may assist dermatopathologists to appropriately diagnose viral infections caused by herpesviruses and papillomaviruses.

Key Words: immunohistochemistry, virus, skin, infection, herpesviruses, papillomaviruses

(*Am J Dermatopathol* 2014;0:1–12)

LEARNING OBJECTIVES

Upon completion of this learning activity, participants should be better able to:

1. Use immunohistochemistry (IHC) to identify viral pathogens that are relevant to dermatopathology.
2. Apply these techniques in the diagnosis of cutaneous viral infections and other related diseases.

INTRODUCTION

Various laboratory techniques can be used to assist in the specific diagnosis of a suspected viral disease. These include light and electron microscopy of a biopsy or smear, serology, viral culture, and immunomorphological methods. Although viral isolation in tissue culture remains the paramount diagnostic method, the development of monoclonal antibodies to various viruses, for use with fluorescent, immunoperoxidase, and enzyme-linked immunosorbent assay techniques, has made possible the rapid diagnosis of many viral infections with a high degree of specificity.[1] These markers cannot replace the routine histologic study; however, they can help to narrow the differential diagnosis.

Immunohistochemistry (IHC) continues to be one of the main adjunctive methods to conventionally stained sections in histopathology. This is mainly related to the fact that it is a relatively simple, fast, and inexpensive method.[2] Because of technical advances, there has been a significant increase in the number of diagnostic immunohistochemical stains available for pathologists and dermatopathologists in recent years. The sensitivity and specificity of certain antibodies, their pattern of staining (nuclear, cytoplasmic, or membranous), and background artifact must be considered in their interpretation. In addition, evaluation must be done in relation to internal control.[3] IHC, however, is only a tool to be used appropriately in the context of clinical and histopathologic correlation; unreasonable use can be misleading and financially cost ineffective.[4]

In this review, we highlight the main immunohistochemical available techniques that have been used and continue to evolve in the diagnosis of mucocutaneous viral infections and discuss their applicability.

IMMUNOHISTOCHEMISTRY

IHC has become an increasingly important histopathologic tool over the past 20 years, and is now a key part of routine practice of dermatopathologists. IHC begins with antigen retrieval by pretreating the tissue to unmask antigens

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All authors and staff in a position to control the content of this CME activity and their spouses/life partners (if any) have disclosed that they have no financial relationships with, or financial interests in, any commercial organizations pertaining to this educational activity.

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hidden by fixatives, such as formalin. This step is often done by microwaving the tissue in citrate buffer or other agents, such as pepsin, proteases, or trypsin. Primary antibody is then applied, which binds the antigens of interest. After washing off the excess primary antibody, a secondary antibody is added to bind the primary antibody. This is then followed by the addition of an enzyme complex, such as avidin–biotin–peroxidase complex, and a chromogen.[2]

Immunoperoxidase techniques involve the use of enzyme labels that convert a colorless substrate into a colored one. The most widely used enzymes are peroxidase and alkaline phosphatase, and the most widely used substrates are diaminobenzidine, amino ethylcarbazole, and nitroblue tetrazolium, which, respectively, produce brown, red, and blue colors. These markers can be attached, or conjugated, directly to the primary antibody or indirectly using secondary antibodies or substances, such as biotin and protein A.[5]

The use of a continuously increasing number of newly raised and commercially available antibodies for IHC has tremendously broadened IHC applicability, this being especially relevant for the diagnosis of different types of infections.[2,4,6] The use of IHC in the diagnosis of cutaneous infections has recently been applied to confirm the diagnosis of specific viral infections that may be difficult to diagnose with certainty using routine microscopy alone.[2,4,7,8] Table 1 lists the main commercially available antibodies for specific viruses that can be useful in dermatopathology.

VIRAL PATHOGENS

Viruses are separated into families on the basis of the type and form of the nucleic acid genome, of the morphological features of the virus particle, and of the mode of replication. There are 4 important families involved in cutaneous viral diseases: the DNA families of Herpesviridae, Papillomaviridae, and Poxviridae, and the RNA family Picornaviridae.[1] In addition to these 4 families, exanthemas can occur in the course of infections with the following families: Adenoviridae, Reoviridae, Togaviridae, Flaviviridae, Retroviridae, Parvoviridae, Paramyxoviridae, Arenaviridae, Filoviridae, and Bunyaviridae.[2,4,7,8] The 3 major DNA families produce lesions that are histologically diagnostic for a disease or group of diseases, whereas the other viruses, particularly the RNA viruses, produce lesions that are often histopathologically nonspecific, usually showing epidermal spongiosis and superficial perivascular lymphohistiocytic infiltrate.

MATERIALS AND METHODS

The biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. For routine histology, 5-μm-thick sections were stained with hematoxylin and eosin. Immunohistochemical staining was performed both at the Pathology Department of Fundación Jiménez Díaz University Hospital in Madrid and the Dermatopathology Laboratory at Friedrichshafen, Germany. Tissue sections were processed on a BioTek Solutions TechMate 500 (Dako) where 1-hour incubation with a primary antibody was performed. The immunohistochemical study of the viral pathogens was performed using the commercially available antibodies described in Table 1. Several antibodies for specific viruses described in the literature were not commercially available at the time of elaboration of the article; therefore, immunohistochemical staining for these pathogens could not be tested.

TABLE 1. Available Antibodies for Specific Viruses Useful in Dermatopathology

Antibody Against	Type/Clone
HSV-1	Rabbit polyclonal
	DAKO
HSV-2	Rabbit polyclonal
	DAKO
VZV	Monoclonal, mouse
	C90.2.8
	Menarini
EBV	Monoclonal, mouse
	CS1-4
	DAKO
CMV	Monoclonal, mouse
	CCH2 + DDG9
	DAKO
HHV-6	Monoclonal/OHV-1, OHV-2, p41
HHV-7	Monoclonal/HHV-7 pp85
HHV-8	Monoclonal, mouse
	13B10
	Menarini
Papillomaviruses (HPV)	HPV L1: monoclonal, mouse
	Cytoimmune diagnostics
	HPV cocktail: monoclonal, mouse
	BPV-1/1H8 + CA
	Zytomed systems
Parvovirus B19 (PVB19)	Monoclonal, mouse
	R92F6
	Menarini
Merkel cell polyomavirus (MCPyV)	Monoclonal, mouse
	CM2B4
	Santa Cruz Biotechnology
SV40 [trichodysplasia spinulosa polyomavirus (TSPyV)]	Monoclonal, mouse
	Pab101
	BD Biosciences
HTLV-1	Monoclonal, mouse
	1A3
	Biozol
Measles	Polyclonal, rabbit
	Acris
Enterovirus	Monoclonal, mouse
	DAKO
ORF *Parapoxvirus* (ORF)	Monoclonal
Ebola virus (EBO)	EBO antigen

HERPESVIRUSES

More than 80 herpesviruses have been identified, 8 of which are known human pathogens. Herpes simplex viruses

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(HSVs) belong to the ubiquitous Herpesviridae family of viruses, which comprises HSV-1, HSV-2, varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV), human herpesviruses (HHV) 6 and 7, and Kaposi sarcoma (KS)–associated HHV-8.

Herpes Simplex Virus and Varicella Zoster Virus

Histopathologic findings of biopsies of fully developed labial and genital vesicles because of HSV and vesicles caused by VZV consist of an intraepidermal blister with varying degrees of epithelial necrosis.[9] Two mechanisms are involved in the formation of intraepidermal vesicles: one is the ballooning of keratinocytes and the other is the reticular degeneration of the epidermis.[9] The most typical changes of the infection are evident in the nuclei of epidermal keratinocytes, where there is peripheral margination of chromatin attached to the nuclear membrane. As a consequence, the keratinocytes show ringed nuclei with a homogeneous ground-glass appearance combined with ballooning of the nucleus. The earliest noteworthy abnormality in the cytoplasm of these keratinocytes is the presence of vacuolization.[9]

The histopathologic differential diagnosis of HSV and VZV infections may be established immunohistochemically. Immunoperoxidase stains specific for HSV-1 (Fig. 1), HSV-2 (Fig. 2), and VZV (Fig. 3) are available commercially.[10,11] In all cases, the cells exhibit both nuclear and cytoplasmic staining, although staining is more intense at the edges of the infected nuclei, confirming the histopathologic observation of cells with peripheral margination of chromatin. The intensity of staining varies between cells depending on the type of infection. In infections by HSV-1 and HSV-2, epidermal keratinocytes show the strongest staining and only occasionally staining of the superficial portion of the follicular infundibulum can be observed. In early infections because of VZV, however, the strongest staining is seen in the cells of the outer root sheath of the hair follicle and in sebocytes of the sebaceous gland.[12,13] IHC can also demonstrate VZV antigens in nerves and other dermal structures. Epidermal keratinocytes, on the contrary, tend to be negative for VZV, at least in the early stages of infection.[13] Based on such immunohistochemical studies and the demonstration of viral antigens in various cutaneous structures, it has been possible to establish the sequence of herpes zoster lesion reactivation.[9] A histopathologic finding of folliculosebaceous involvement is characteristic of incipient erythematous lesions, whereas epidermal involvement and the formation of vesicles will be found in fully developed lesions.[9,13]

Clinicians and pathologists may also wish to use laboratory tests to establish definitive diagnosis in the case of atypical cutaneous manifestations of these viruses. For example, IHC was able to identify HSV infection in 5 bedridden geriatric patients (type 1 in 3 and type 2 in 2) with genital ulcers when histology was suggestive of HSV infection in only 2 of the 5 patients.[14,15] Another study showed that the sensitivity and specificity of IHC were comparable with in situ hybridization (ISH) in diagnosing HSV infections.[16] Similarly, IHC has also been shown to have higher specificity and sensitivity than standard microscopic assessments in diagnosing VZV infection through detection of VZV ORF63 encoded protein (IE63) and VZV late protein gE on both smears and formalin-fixed paraffin-embedded skin sections.[10] This can be of special significance in allowing early diagnosis of VZV infection in immunocompromised patients and thus early treatment. VZV infections may also

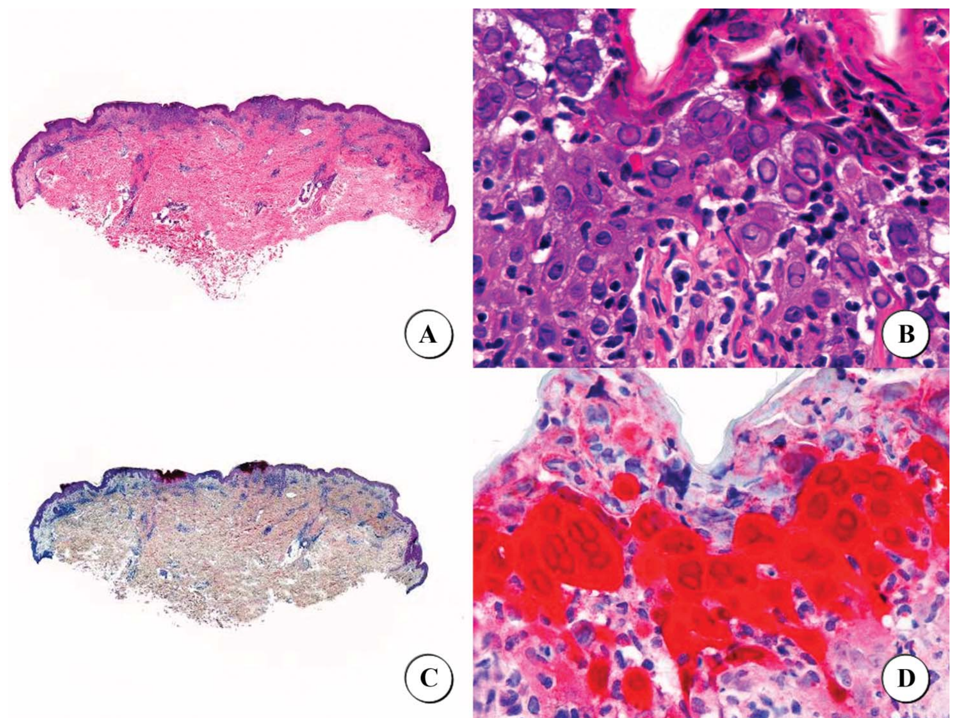

FIGURE 1. Histopathologic and immunohistochemical findings in a cutaneous infection by HSV-1 in the upper lip. A, Scanning power showing 2 foci of epidermal involvement and inflammatory infiltrate in the superficial dermis. B, Higher magnification demonstrated the characteristic cytopathic effect of herpesvirus infections in epidermal keratinocytes. C, The same case immunohistochemically studied for HSV-1 antibody. D, Positivity in the nuclei and cytoplasm of many epidermal keratinocytes. (A and B, hematoxylin–eosin stain; C and D, IHC for HSV-1. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

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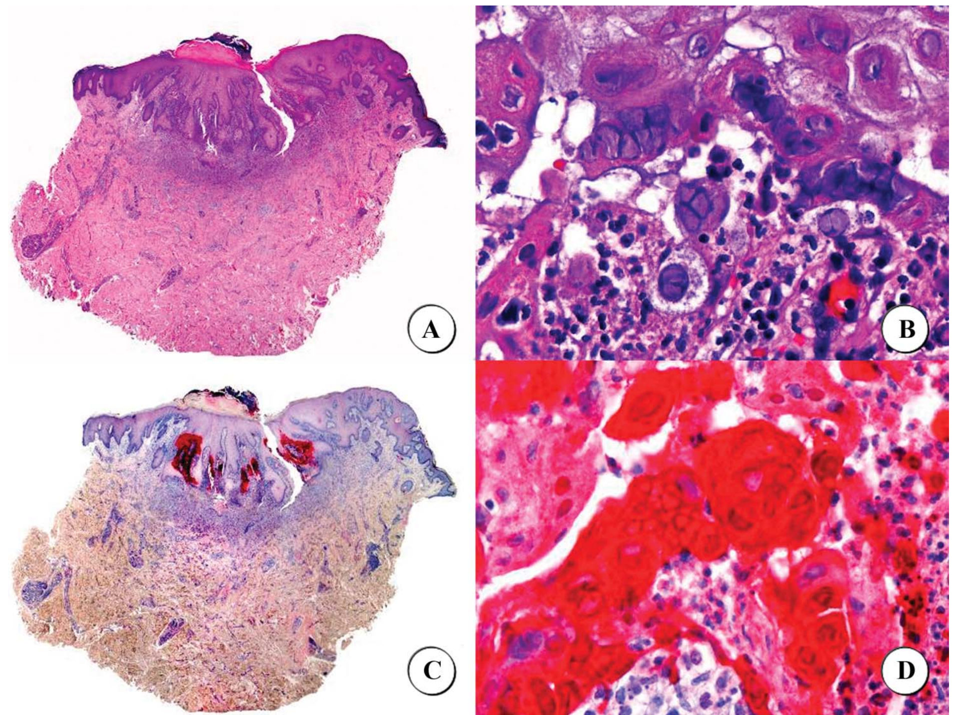

FIGURE 2. Histopathologic and immunohistochemical findings in a persistent cutaneous infection by HSV-2 in genital skin of a patient with AIDS. A, Scanning power showing irregular epidermal hyperplasia. B, Higher magnification demonstrated the characteristic cytopathic effect of herpesvirus infections in epidermal keratinocytes. C, The same case immunohistochemically studied for HSV-2 antibody. D, Positivity in the nuclei and cytoplasm of many epidermal keratinocytes. (A and B, hematoxylin–eosin stain; C and D, IHC for HSV-2. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

be differentiated with certainty from HSV infections using monoclonal antibodies against the VZV envelope glycoprotein gp1.[17]

Epstein–Barr Virus

EBV, or HHV-4, is a DNA γ-herpes virus. The DNA core is surrounded by an icosahedral nucleocapsid of 162 capsomers and the envelope. The EBV genome encodes for nearly 100 viral proteins. This genome exists in 2 forms: circular (episomal) and lineal. The episomal form is the one adopted in latency periods, whereas the lineal form is the one evidenced during the lytic phase in which EBV replicates.

EBV causes infectious mononucleosis, hairy leukoplakia in patients with AIDS, Lipschütz ulcer (ulcus vulvae acutum),

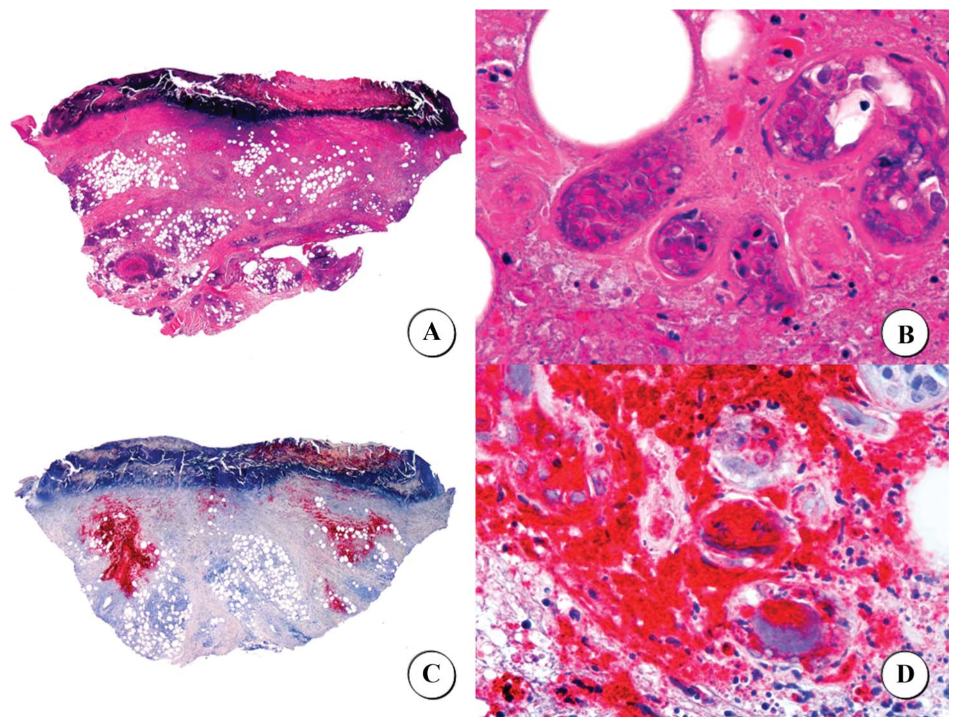

FIGURE 3. Figure 1. Histopathologic and immunohistochemical findings in a necrotic herpes zoster infection involving the lower back in a patient with AIDS. A, Scanning power showing epidermal necrosis and inflammatory infiltrate involving the upper and lower dermis. B, Higher magnification demonstrated necrotic eccrine coils. C, The same case immunohistochemically studied for VZV antibody showing several foci of positivity in the dermis. D, Positivity in the nuclei and cytoplasm of many necrotic keratinocytes of the eccrine coils. (A and B, hematoxylin–eosin stain; C and D, IHC for VZV. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

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a variety of benign and malignant lymphoproliferative processes, and nasopharyngeal lymphoepithelial carcinoma.[9,17,18] In immunosuppressed patients, mainly HIV-positive and transplanted ones, an association between myoid tumors and EBV has been well established. The spectrum of EBV-associated myoid tumors includes leiomyoma, smooth muscle tumor of uncertain malignant potential, leiomyosarcoma, and myopericytoma. In general, EBV-related myoid tumors show some clinical peculiarities, such as being multifocal and appearing in uncommon locations.[19–28] EBV-associated leiomyosarcoma shows a more indolent biological behavior than classical leiomyosarcomas.[24]

In dermatology, it is also common to see a maculopapular rash in patients with infectious mononucleosis who have been treated with ampicillin, because of the presence of immunoglobulin (Ig) G or IgM antibodies against penicillin, but not IgE antibodies, as is the case in the hypersensitivity reaction to penicillin. As mentioned above, histopathologic findings in these rashes are nonspecific.

EBV displays a very high seroprevalence and is characterized by its latent persistence in memory B lymphocytes. The mere detection of EBV DNA therefore lacks diagnostic relevance. Switching from latent to replicative infection has only exceptionally been reported in B lymphocytes, and such a lytic phenomenon mainly occurs in the epithelia.

There are commercialized antibodies against latent membrane protein (LMP) 1 detected by conventional IHC and against Epstein–Barr nuclear antigen (EBNA) 2 detected by immunofluorescence, but these methods have relatively low detection sensitivity and accuracy. In contrast, identifying Epstein-Barr–encoded RNAs (EBERs) by ISH facilitates viral detection with high sensitivity and specificity and allows the visualization of viral RNA in the nucleus of tumor cells. At the present time, there are commercially available probes that can be used on paraffin-embedded tissue with a permanent chromogen. Therefore, ISH remains the gold standard for EBV detection in tissue samples[17] (Fig. 4). Western blot, flow cytometry, and enzyme-linked immunosorbent assay can potentially detect and measure selected viral proteins for which antibodies are available. However, the single most informative protein-based assay is ISH because it permits localization of protein in the context of histopathology, facilitating assessment of the medical significance of the infection.[29] Localization is achievable in paraffin-embedded sections for latent and lytic viral factors, including EBNA1, EBNA2, LMP1, LMP2, BHRF1, BZLF1, and BRLF1.[30] Interpretation of results, defining the spectrum of expressed genes and their localization to benign- or malignant-appearing cells, complements EBER ISH for diagnosis of EBV-related disease.[31] Immunostaining with EBV-LMP yields a cytoplasmic pattern.[12] This marker is useful in the diagnosis of posttransplant lymphoproliferative disorders, Hodgkin lymphoma, and other lymphomas. It is also positive in 25%–50% of nasopharyngeal carcinomas.[12] LMP1 immunostain (cytoplasmic and membranous localization) has been shown to be nearly as effective as EBER ISH in identifying EBV in lymph nodes of patients with infectious mononucleosis. Likewise, BZLF1 (characterizes lytic viral replication) immunostains (nuclear staining) have been shown to be useful in confirming diagnosis of oral hairy leukoplakia in tongue biopsies of AIDS patients.[32]

Technical problems can foil interpretation of immunohistochemical results. For example, EBNA1 is thought to be expressed in virtually all latently infected tumors; yet, EBNA1

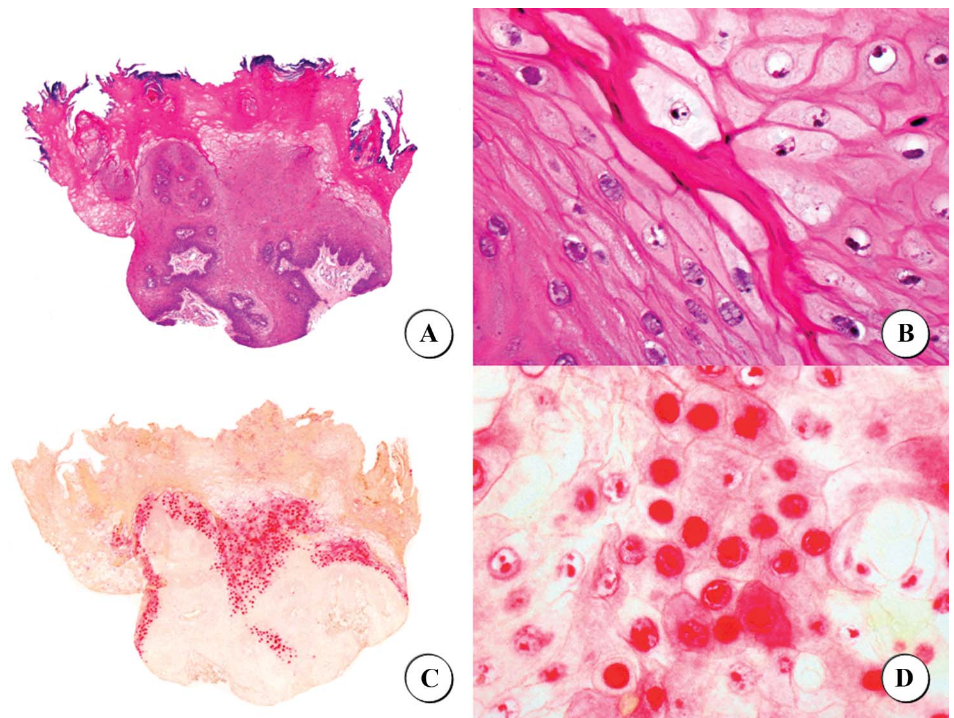

FIGURE 4. Histopathologic and ISH findings in a patient with hairy leukoplakia and AIDS. A, Irregular epithelial hyperplasia with ballooning of the keratinocytes of the upper layers. B, Higher magnification showing large pale-staining cells in the upper layers of the epithelium with irregular wrinkled nuclei. Note the presence of some intranuclear inclusion bodies. C, The same case studied by ISH for EBER. D, Many nuclei of the keratinocytes of the upper layers show EBER positivity. (A and B, hematoxylin–eosin stain; C and D, ISH for EBER. Original magnifications: A, ×10; B, ×400; C, ×10, D, ×400.)

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IHC is not sensitive enough to reliably substitute for EBER ISH. Furthermore, the 2B4 clone of EBNA1 antibody cross-reacts with human MAGEA4, potentially causing false-positive interpretations.[33] Problems such as these emphasize the need to validate laboratory assays before they are used in clinical investigations.

Cytomegalovirus

CMV belongs to the subgroup of β-herpesviruses. Like other members of the family Herpesviridae, this virus produces primary infection, latent infection, and reinfection; however, its site of latency is not known. CMV infection is especially common in immune-compromised patients (such as HIV-infected patients), and the cutaneous manifestations can be quite variable, including ulcers, vesicles, papules, purpuric macules, verrucous lesions, prurigo nodularis–like lesions, and digital infarcts. In CMV infections, the characteristic observations are made in the endothelial cells of dermal blood vessels. The nuclei of these cells have large eosinophilic inclusions surrounded by a clear halo.[34] Less frequently, inclusions similar to those found in the nuclei of endothelial cells have also been described in the nuclei of fibroblasts and macrophages and also in the nuclei of the epithelial cells of eccrine ducts in CMV infections.[35]

CMV skin infection may be difficult to identify by clinical or histopathologic findings for various reasons. First, cutaneous CMV is rare, and the paucity of cases does not lend itself to microscopists having the opportunity to study a large number of cases. Second, the clinical manifestations of CMV infection vary, and there are no specific clinical findings that allow a clinician to suspect the right diagnosis. Third, the histopathologic findings of cutaneous CMV are typically present in scattered cells, and often, only a few cells have cytopathologic changes.[35,36] Therefore, immunohistochemical analysis may be needed to confirm the diagnosis in the setting of intense inflammation or in cases where only few cells are infected[35] (Fig. 5). IHC allows for rapid diagnosis; its sensitivity is higher than that of light microscopy and is comparable with that of culture and ISH.

Immunostaining with monoclonal anti-CMV antibody by immunoperoxidase methods gives a nuclear staining pattern in early-stage infections and a nuclear and cytoplasmic pattern in late-stage infections.[12] The anti-CMV antibody is known to bind with great avidity to CMV antigen synthesized in both early and late stages of infection.[37]

Human Herpesviruses 6 and 7

HHV-6 and HHV-7 are ubiquitous and have been associated with several cutaneous diseases, including roseola infantum, infectious mononucleosis, Rosai–Dorfman disease, pityriasis rosea, lichen planus, hypersensitivity reactions, graft-versus-host disease, and several other cutaneous manifestations, including various malignancies.[38] HHV-6 consists of 2 closely related yet distinct viruses, designated HHV-6 variant A and HHV-6 variant B. HHV-6 variant B is the etiological agent of the common childhood illness exanthema subitum, also known as roseola infantum or sixth disease of childhood. In adults, infection is seen primarily in immunocompromised individuals. In immunocompetent patients, the

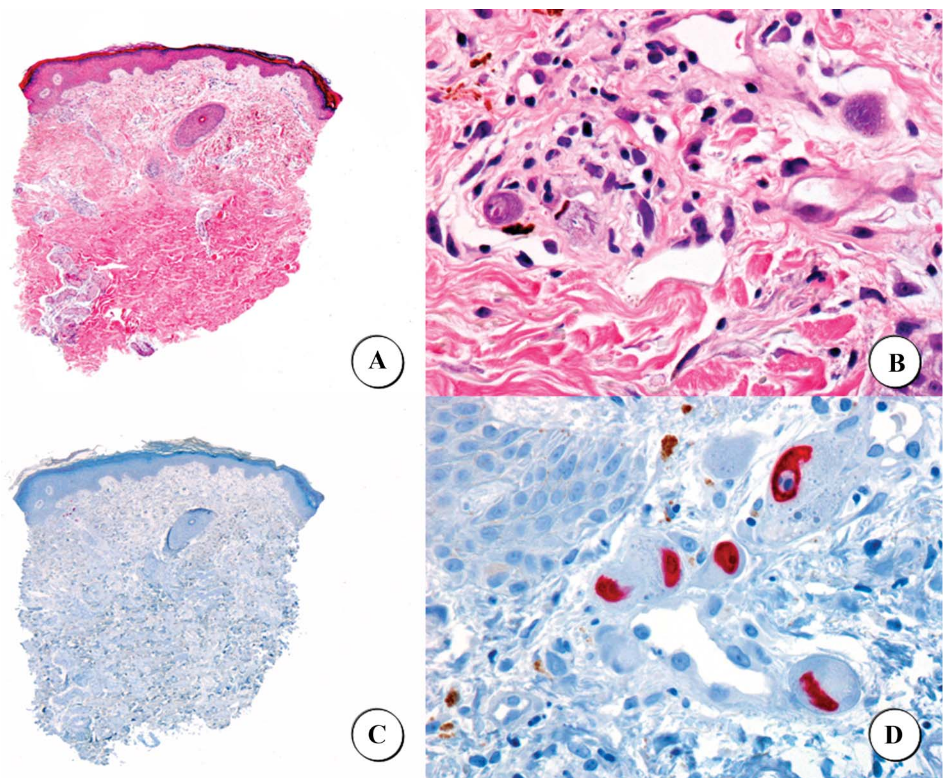

FIGURE 5. Histopathologic and immunohistochemical findings in the border of a perianal ulcer in a HIV-infected patient. A, Scanning power showing discrete perivascular infiltrates in superficial dermis and around eccrine units. B, Higher magnification demonstrated that the nuclei of some endothelial cells and perivascular cells contained large eosinophilic inclusions surrounded by a clear halo. C, The same case immunohistochemically studied for CMV. D, Immunoexpression for CMV in the cells containing eosinophilic inclusions in their nuclei. (A and B, hematoxylin–eosin stain; C and D, IHC for CMV. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

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infection is usually diagnosed on the basis of its clinical features. However, the diagnosis in immunocompromised patients may need additional laboratory methods, including viral culture, serologic testing, polymerase chain reaction (PCR) assay, and tissue biopsy.[38] The histopathologic findings of HHV-6 and HHV-7 cutaneous infections are nonspecific. Epidermal spongiosis, some degree of vacuolar degeneration of the basal layer of the epidermis, and a superficial perivascular lymphocytic infiltrate may be observed.[9]

Immunohistochemical detection of HHV-6 and HHV-7 in skin biopsies is not commonly performed in routine clinical practice. However, several studies have reported positive results in the course of different clinical settings. Sumiyoshi et al[39] reported positive immunohistochemical staining with the HHV-6–specific monoclonal antibodies OHV-1 and OHV-2, using the avidin–biotin alkaline phosphatase method, in skin biopsy specimens from patients with primary HHV-6 infection and erythroderma. They found positive staining in the lymphocytes infected with HHV-6 in the skin.[39] Yadav et al[40] also reported the detection of HHV-6–associated antigens in formalin-fixed and paraffin-embedded oral tissues from patients with lichen planus, leukoplakia, and squamous cell carcinoma. They used the mouse (6A5G3) monoclonal antibody to the HHV-6 gp116/64/54 KDa component, which is a late protein in the viral replicative cycle. Furthermore, Broccolo et al[41] reported the immunohistochemical detection of the HHV-7 pp85 and the HHV-6 p41 antigens in skin biopsy specimens of patients with pityriasis rosea (17% and 67% of patients analyzed, respectively), suggesting that they might play an etiological role in this disease. HHV-7 and HHV-6 antigen-positive cells were located mainly in the perifollicular and papillary dermis, with the exception of 1 patient in whom HHV-7 pp85 was also found in the epidermal cells.

Human Herpesvirus 8

HHV-8 has been shown to be involved in the development of all the epidemiologic variants of KS. This involvement was demonstrated in 1994 when unique DNA sequences were isolated from biopsies of KS.[42] The virus, initially called KS-associated herpesvirus, was subsequently renamed HHV-8. HHV-8 infects the endothelial-derived spindle cells of KS and $CD19^{+}$ B cells. This latter event may be etiologically significant in the causation of some cases of multicentric Castleman disease and primary effusion lymphoma.[43] It has also been found in lymphomas and in other lymphoproliferative disorders with heterogeneous presentations.[44] Its presence in a number of skin cancers and in lesions of pemphigus vulgaris and pemphigus foliaceus has not been satisfactorily explained, although tropism for lesional skin has been postulated.[45,46]

HHV-8 can be detected by PCR in paraffin-embedded tissue, although the viral load seems to be low.[47,48] It is much easier to perform IHC using the commercially available monoclonal antibody against HHV-8 that is directed against the C-terminus of the latent nuclear antigen-1 (LNA-1) encoded by the ORF-73 gene.[49] The presence of HHV-8 in the nuclei of proliferating cells in KS lesions may be demonstrated in all its epidemiologic variants and from the earliest phases of the process. It is largely confined to the spindle cells in the nodular phase (Fig. 6), but it is found in the endothelial cells of the slit-like vessels in the early patch and plaque stages.

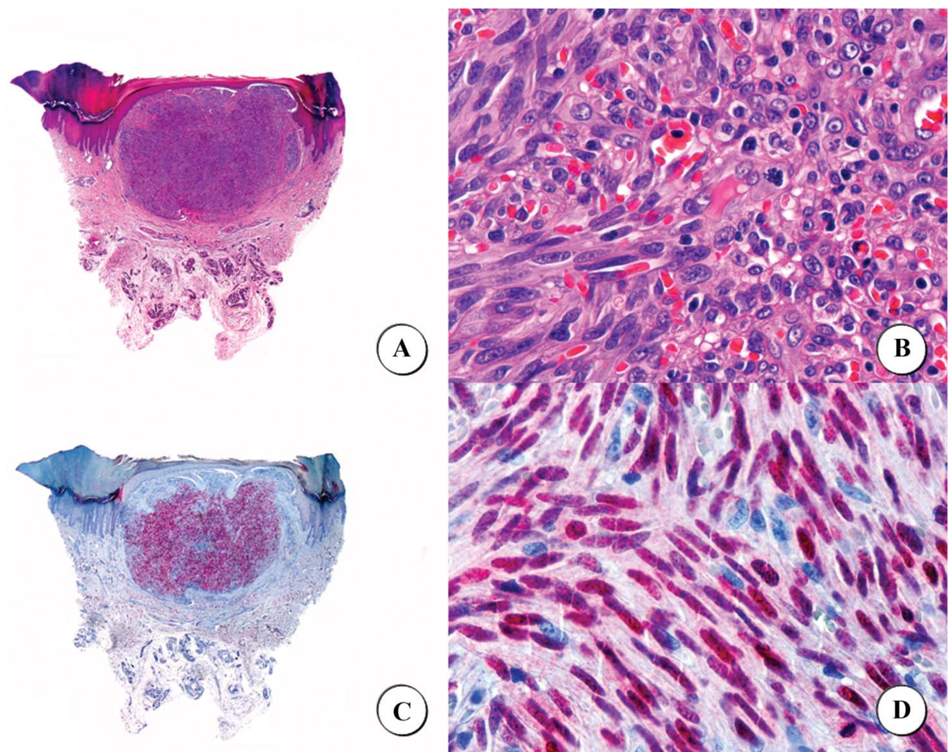

FIGURE 6. Histopathologic and immunohistochemical findings in a nodular lesion of an elderly male with classic KS. A, Scanning power showing a well-circumscribed dermal nodule. B, Higher magnification demonstrated short fascicles of spindled or rounded cells (depending on the cut plane) and small spaces filled by red cells. C, The same case immunohistochemically studied for HHV-8. D, Immunoexpression for HHV-8 in most of the nuclei of spindled cells. (A and B, hematoxylin–eosin stain; C and D, IHC for HHV-8. Original magnifications: A, ×10; B, ×400; C, ×10; D ×400.)

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HHV-8 has both a lytic and latent phase of replication. During the latent phase, viral DNA copies are maintained as episomes attached to the host chromosome with the help of LNA-1.[50] This explains why LNA-1 immunoreactivity in KS cells most often appears as stippled nuclear staining. Most cells in KS lesions and HHV-8–infected cell cultures are latently infected. In only a small percentage (<5%) of infected cells is lytic HHV-8 replication seen.[51] This is clearly helpful for diagnostic purposes because most KS lesional cells will accordingly be LNA-1 immunoreactive.

Given the strong association between HHV-8 infection and KS, positive immunoreactivity for LNA-1 has proved to be the most useful diagnostic marker to help differentiate KS from its mimickers, including angiosarcoma, kaposiform hemangioendothelioma, spindle cell hemangioma, and benign lymphangioendothelioma, among others.[52,53] However, a minority of conventional angiosarcomas (29%) were found to be HHV-8 positive,[54] whereas no HHV-8 expression could be observed in radiation-related angiosarcomas.[55] LNA-1 IHC is favored over PCR (for HHV-8) for the evaluation of problematic vascular proliferations in patients infected with HIV. LNA-1 is a highly sensitive and specific immunohistochemical marker for KS in both the general (HIV negative) population and HIV-positive patients.[56] HHV-8 immunoreactivity seems to be unaffected by HIV status, patient age, gender, tumor recurrence, or the site of the KS lesion.[49]

Immunostaining KS with LNA-1 for diagnostic purposes is especially useful for the following: (1) Early subtle patch-stage lesions in which the histopathologic findings are not immediately obvious and may be difficult to diagnose on tissue biopsy. In these cases, inflammatory processes are the main differential diagnosis to be considered, stasis dermatitis being the most important. Other histopathologic alterations, such as ulceration, lymphedema, and secondary infections, may obscure the histopathologic characteristic of KS, rendering viral detection crucial for the diagnosis. (2) KS lesions that mimic other conditions, like pyogenic granuloma–like and angiomatous variants of KS. These histopathologic variants of KS are characterized by the proliferation of well-formed capillaries organized in lobules and surrounded by epidermal collarets, with no solid aggregates of fusiform cells, and they mimic benign vascular proliferations with a lobular capillary hemangioma pattern.[50] (3) KS presenting in unusual locations. (4) Histopathologic differential diagnosis with other vascular proliferations mimicking KS, such as hobnail hemangioma, spindle cell hemangioma, benign lymphangioendothelioma, and kaposiform hemangioendothelioma. The presence of HHV-8, however, seems not to be fully restricted to KS, as HHV-8 has been detected (albeit rarely) in some angiosarcomas, hemangiomas, and dermatofibromas,[57] although the meaning of this finding remains uncertain. Finally, the immunohistochemical demonstration of HHV-8 DNA may be a useful adjunct in the diagnosis of KS by fine-needle aspiration.[58]

PAPILLOMAVIRUSES

Papillomaviruses are DNA viruses, which replicate in the nucleus. The only important virus in this group in dermatopathology is the human papillomavirus (HPV), which produces various types of warts on different areas of the skin.[59] Several phylogenetic groups may be distinguished among the more than 150 known HPV types. Group A HPV types, occurring on genitals and mucous membranes, and cutaneous group B beta HPV, also associated with epidermodysplasia verruciformis, are of greatest significance for dermatologists.

The diagnosis of HPV-associated benign and malignant epithelial lesions is predominantly based on clinical features and conventional histopathology. Two of the most specific histopathologic findings are the presence of koilocytosis, which is not invariable, especially in long-standing warts, and the so-called koilocytotic atypia, mostly seen at the granular layer, which consists of nuclear variation in size and staining pattern, with irregularity of nuclear membrane and binucleated or multinucleated cells.[60] With only few exceptions (HPV types 4 and 63 with filamentous inclusion bodies[61] and HPV type 60 with eosinophilic intracytoplasmic large granules[62]), histology does not grant exact specification of the HPV type involved.[9] Papillomavirus antigen can be detected by immunoperoxidase methods.[63] Immunohistochemical detection of the HPV L1 capsid antigen (Fig. 7) is indicative of replicative HPV infections, but this method does not facilitate identification of the virus type involved, either. HPV antigens are detected in 64% of acanthopapillomas with koilocytes, but not in any lesion without histologic evidence for koilocytes, thus indicating that IHC is only of limited value.[64]

The use of new techniques, such as DNA hybridization, has allowed the separation of more than 120 antigenically distinct strains of HPV.[65,66] Further genotypes have been identified, but not fully characterized. PCR is now used routinely for the typing of HPV.[67] In recent years, attempts have been made to relate specific antigenic strains of HPV to particular clinicopathologic groups of verrucae.[68,69]

In the field of cervical cancer, the identification of HPV-related precancer that is likely to progress to an invasive carcinoma is very important. Although cervical cancer screening relies on cervical cytology and high-risk HPV detection, the histologic diagnosis, and specifically lesion grade, is the main parameter that drives clinical management of screen-positive women. Morphologically diagnosed squamous intraepithelial lesions regress spontaneously in more than half of the cases, but identifying those likely to persist and progress is not currently possible based on morphology. It has been suggested that patterns of viral protein expression may be used to differentiate between self-limited productive viral infection and a true precancer.[70] Some studies have evaluated the immunohistochemical expression of HPV capsid proteins L1 and L2 in squamous intraepithelial lesions as a predictive tool for progression.[71] Lack of major capsid protein L1 expression has been suggested as a feature in progressive lesions, whereas expression of the minor capsid protein L2 has not been extensively evaluated.

Recently, Wititsuwannakul et al[72] have evaluated the sensitivity and specificity of a commercially available anti-HPV antibody (BPV-1/1H8 + CAMVIR, ab 2417, Abcam),

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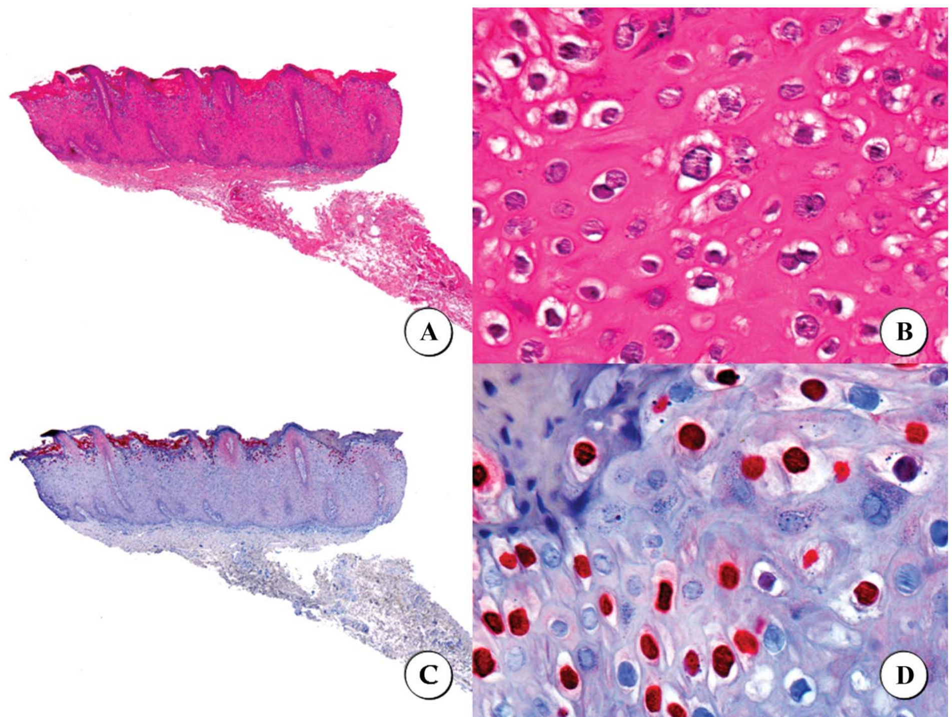

FIGURE 7. Histopathologic and immunohistochemical findings in a genital wart from an otherwise healthy young adult male. A, Scanning power showing a regular acanthosis of the epithelium. B, Higher magnification showed prominent koilocytosis in the upper layers of the epithelium. C, The same case immunohistochemically studied for HPV L1 capsid antigen. D, Immunoexpression for HPV L1 is seen in many nuclei of the keratinocytes of the upper layers. (A and B, hematoxylin–eosin stain; C and D, IHC for HPV L1. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

which detects HPV types 1, 6, 11, 16, 18, and 31 in formalin-fixed paraffin-embedded tissue, in 25 lesions (both HPV induced and non-HPV induced) mostly from the genital region, compared with ISH and hematoxylin and eosin staining. They found a sensitivity and specificity for this anti-HPV antibody of 90.9% and 85.7%, respectively, compared with ISH. IHC, like ISH, was generally positive in cases showing koilocytes/koilocytotic atypia (86%). However, IHC also detected productive infection with HPV in 23% (3 of 13) of cases without koilocytes/koilocytotic atypia. Positive immunostaining is mostly seen in corneocytes, the nuclei of the keratinocytes of the granular layer, and upper layers of the epidermis (Fig. 8). A

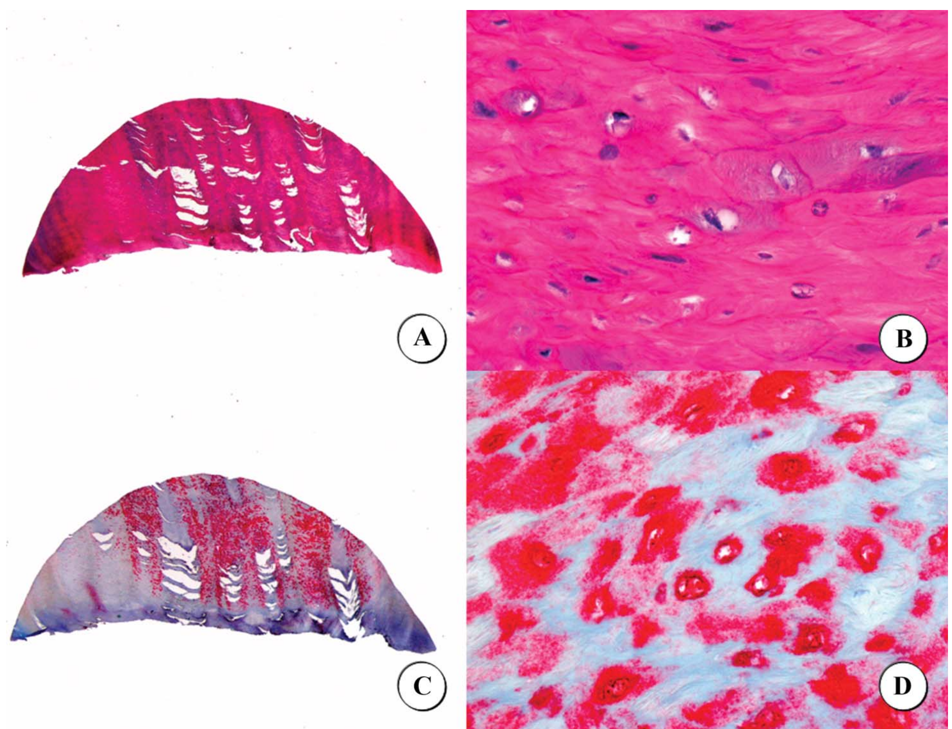

FIGURE 8. Histopathologic and immunohistochemical findings in a genital wart from a young adult woman with history of persistent condyloma acuminatum involving the vulva. A, Scanning power of a shave biopsy showed a thick compact orthokeratotic horny layer. B, Higher magnification showed some koilocytes in the upper layers of the horny layer. C, The same case immunohistochemically studied with CAMVIR antibody. D, Immunoexpression for HPV in many nuclei of the corneocytes of the upper layers with CAMVIR antibody. (A and B, hematoxylin–eosin stain; C and D, IHC for CAMVIR antibody. Original magnifications: A, ×10; B, ×400; C, ×10; D, ×400.)

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major disadvantage of IHC, compared with ISH and PCR, is its inability to type HPV. But IHC shows considerable advantages over ISH and PCR, such as the low cost, rapid turnaround time, and the ability to visualize productive HPV infection in tissue sections.[72] Because results of IHC for HPV with this antibody correlate with ISH, both tests are very helpful to confirm HPV infection (IHC) and subsequent HPV typing (ISH).

In conclusion, this review is focused on the use of IHC to identify viral pathogens that are relevant to dermatopathology and on the usefulness of these techniques in the diagnosis of cutaneous viral infections and other related diseases.

REFERENCES

1. Weedon D. Viral diseases. In: Weedon D, ed. *Weedon's Skin Pathology*. 3rd ed. Philadelphia, PA: Churchil Livingstone Elsevier; 2010:608–631.
2. Abbas O, Bhawan J. Infections in Dermatopathology: emerging frontiers. *Am J Dermatopathol.* 2012;34:789–796.
3. Hoang MP. Role of immunohistochemistry in diagnosing tumors of cutaneous appendages. *Am J Dermatopathol.* 2011;33:765–771.
4. Wasserman J, Maddox J, Racz M, et al. Update on immunohistochemical methods relevant to dermatopathology. *Arch Pathol Lab Med.* 2009;133: 1053–1061.
5. Fuertes L, Santonja C, Kutzner H, et al. Immunohistochemistry in dermatopathology: a review of the most commonly used antibodies (part I). *Actas Dermosifiliogr.* 2013;104:99–127.
6. Braun-Falco M, Schempp W, Weyers W. Molecular diagnosis in dermatopathology: what makes sense, and what doesn't. *Exp Dermatol.* 2009;18:12–23.
7. Cherry JD. Viral exanthemas. *Dis Mon.* 1982;28:1–56.
8. Lupi O, Tyring SK. Tropical dermatology: viral tropical diseases. *J Am Acad Dermatol.* 2003;49:979–1000.
9. Requena L, Requena C. Histopathology of the more common viral skin infections [in Spanish]. *Actas Dermosifiliogr.* 2010;101:201–216.
10. Nikkels AF, Debrus S, Sadzot-Delvaux C, et al. Immunohistochemical identification of varicella-zoster virus gene 63-encoded protein (IE63) and late (gE) protein on smears and cutaneous biopsies: implications for diagnostic use. *J Med Virol.* 1995;47:342–347.
11. Oda Y, Okada Y, Katsuda S, et al. Immunohistochemical study on the infection of herpes simplex virus, human cytomegalovirus, and Epstein-Barr virus in secondary diffuse interstitial pneumonia. *Hum Pathol.* 1994; 25:1057–1062.
12. Fuertes L, Santonja C, Kutzner H, et al. Immunohistochemistry in dermatopathology: a review of the most commonly used antibodies (part II). *Actas Dermosifiliogr.* 2013;104:181–203.
13. Walsh N, Boutilier R, Glasgow D, et al. Exclusive involvement of folliculosebaceous units by herpes. A reflection of early herpes zoster. *Am J Dermatopathol.* 2005;27:189–194.
14. Eyzaguirre E, Haque AK. Application of immunohistochemistry to infections. *Arch Pathol Lab Med.* 2008;132:424–431.
15. Nikkels AF, Pierard GE. Perineal herpes simplex infection in bedridden geriatric patients. *Am J Clin Dermatol.* 2007;8:79–83.
16. Nikkels AF, Debrus S, Sadzot-Delvaux C, et al. Comparative immunohistochemical study of herpes simplex and varicella-zoster infections. *Virchows Arch a Pathol Anat Histopathol.* 1993;422:121–126.
17. Kempf W, Flaig MJ, Kutzner H. Molecular diagnostics in infectious skin diseases. *J Dtsch Dermatol Ges.* 2013;11:50–58.
18. Fernández Flores A. Epstein-Barr virus in cutaneous pathology. *Am J Dermatopathol.* 2013;35:763–786.
19. Orlow SJ, Kamino H, Lawrence RL. Multiple subcutaneous leiomyosarcomas in an adolescent with AIDS. *Am J Pediatr Hematol Oncol.* 1992; 14:265–268.
20. van Gelder T, Vuzevski VD, Weimar W. Epstein-Barr virus in smooth muscle tumors. *N Engl J Med.* 1995;332:1719.
21. Cheuk W, Li PC, Chan JK. Epstein-Barr virus-associated smooth muscle tumour: a distinctive mesenchymal tumour of immunocompromised individuals. *Pathology*. 2002;34:245–249.
22. Chang JY, Wang S, Hung CC, et al. Multiple Epstein-Barr virus-associated subcutaneous angioleiomyomas in a patient with acquired immunodeficiency syndrome. *Br J Dermatol.* 2002;147:563–567.
23. Suankratay C, Shuangshoti S, Mutirangura A, et al. Epstein-Barr virus infection-associated smooth-muscle tumors in patients with AIDS. *Clin Infect Dis.* 2005;40:1521–1528.
24. Deyrup AT, Lee VK, Hill CE, et al. Epstein-Barr virus-associated smooth muscle tumors are distinctive mesenchymal tumors reflecting multiple infection events: a clinicopathologic and molecular analysis of 29 tumors from 19 patients. *Am J Surg Pathol.* 2006;30:75–82.
25. Khunamornpong S, Sukpan K, Suprasert P, et al. Epstein-Barr virus-associated smooth muscle tumor presenting as a vulvar mass in an acquired immunodeficiency syndrome patient: a case report. *Int J Gynecol Cancer.* 2007;17:1333–1337.
26. Gallien S, Zuber B, Polivka M, et al. Multifocal Epstein-Barr virus associated smooth muscle tumor in adults with AIDS: case report and review of the literature. *Oncology*. 2008;74:167–176.
27. Petersson F, Huang J. Epstein-Barr virus–associated smooth muscle tumor mimicking cutaneous angioleiomyoma. *Am J Dermatopathol.* 2011;33:407–409.
28. Ramdial PK, Sing Y, Deonarain J, et al. Dermal Epstein Barr virus–associated leiomyosarcoma: tocsin of acquired immunodeficiency syndrome in two children. *Am J Dermatopathol.* 2011;33:392–396.
29. Gulley ML, Tang W. Laboratory assays for Epstein-Barr virus-related disease. *J Mol Diagn.* 2008;10:279–292.
30. Niedobitek G, Herbst H. In situ detection of Epstein-Barr virus and phenotype determination of EBV-infected cells. *Methods Mol Biol.* 2006;326:115–137.
31. Delecluse H-J, Feederle R, O'Sullivan B, et al. Epstein-Barr virus-associated tumours: an update for the attention of the working pathologist. *J Clin Pathol.* 2007;60:1358–1364.
32. Young LS, Lau R, Rowe M, et al. Differentiation-associated expression of the Epstein-Barr virus BZLF1 transactivator protein in oral hairy leukoplakia. *J Virol.* 1991;65:2868–2874.
33. Hennard C, Pfuhl T, Buettner M, et al. The antibody 2B4 directed against the Epstein-Barr virus (EBV)-encoded nuclear antigen 1 (EBNA1) detects MAGE-4: implications for studies on the EBV association of human cancers. *J Pathol.* 2006;209:430–435.
34. Walker JD, Chesney TM. Cytomegalovirus infection of the skin. *Am J Dermatopathol.* 1982;4:263–265.
35. Resnik KS, DiLeonardo M, Maillet M. Histopathologic findings in cutaneous cytomegalovirus infection. *Am J Dermatopathol.* 2000;22:397–407.
36. Daudén E, Fernández-Buezo G, Fraga J, et al. Mucocutaneous presence of cytomegalovirus associated with human immunodeficiency virus infection: discussion regarding its pathogenetic role. *Arch Dermatol.* 2001;137:443–448.
37. Plachter B, Nordin M, Zweyberg Wingart B, et al. The DNA-binding protein p52 of human cytomegalovirus reacts with monoclonal antibody CCH2 and associates with the nucleolar membrane at late times after infection. *Virus Res.* 1992;24:265–276.
38. Wolz MM, Sciallis GF, Pittelkow MR. Human herpesviruses 6, 7, and 8 from a dermatologic perspective. *Mayo Clin Proc.* 2012;87:1004–1014.
39. Sumiyoshi Y, Akashi K, Kikuchi M. Detection of human herpes virus 6 (HHV 6) in the skin of a patient with primary HHV 6 infection and erythroderma. *J Clin Pathol.* 1994;47:762–763.
40. Yadav M, Arivananthan M, Chandrashekran A, et al. Human herpesvirus-6 (HHV-6) DNA and virus-encoded antigen in oral lesions. *J Oral Pathol Med.* 1997;26:393–401.
41. Broccolo F, Drago F, Careddu AM, et al. Additional evidence that pityriasis rosea is associated with reactivation of human herpesvirus-6 and -7. *J Invest Dermatol.* 2005;124:1234–1240.
42. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science*. 1994; 266:1865–1869.
43. Laurent C, Meggetto F, Brousset P. Human herpesvirus 8 infections in patients with immunodeficiencies. *Hum Pathol.* 2008;39:983–993.
44. Du M-Q, Bacon CM, Isaacson PG. Kaposi sarcoma-associated herpesvirus/human herpesvirus 8 and lymphoproliferative disorders. *J Clin Pathol.* 2007;60:1350–1357.
45. Nishimoto S, Inagi R, Yamanishi K, et al. Prevalence of human herpesvirus-8 in skin lesions. *Br J Dermatol.* 1997;137:179–184.
46. Memar OM, Rady PL, Goldblum RM, et al. Human herpesvirus 8 DNA sequences in blistering skin from patients with pemphigus. *Arch Dermatol.* 1997;133:1247–1251.

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47. Cathomas G, McGandy CE, Terracciano LM, et al. Detection of herpesvirus-like DNA by nested PCR on archival skin biopsy specimens of various forms of Kaposi sarcoma. *J Clin Pathol*. 1996;49:631–633.
48. Bezold G, Messer G, Peter RU, et al. Quantitation of human herpes virus 8 DNA in paraffin-embedded biopsies of HIV-associated and classical Kaposi's sarcoma by PCR. *J Cutan Pathol.* 2001;28:127–130.
49. Hong A, Davies S, Lee CS. Immunohistochemical detection of the human herpes virus 8 (HHV8) latent nuclear antigen-1 in Kaposi's sarcoma. *Pathology*. 2003;35:448–450.
50. Pantanowitz L, Otis CN, Dezube BJ. Immunohistochemistry in Kaposi's sarcoma. *Clin Exp Dermatol.* 2010;35:68–72.
51. Douglas JL, Gustin JK, Dezube BJ, et al. Kaposi's sarcoma: a model of both malignancy and chronic inflammation. *Panminerva Med.* 2007;49:119–138.
52. Cheuk W, Wong KO, Wong CS, et al. Immunostaining for human herpesvirus 8 latent nuclear antigen-1 helps distinguish Kaposi sarcoma from its mimickers. *Am J Clin Pathol.* 2004;121:335–342.
53. Robin YM, Guillou L, Michels JJ, et al. Human herpesvirus 8 immunostaining: a sensitive and specific method for diagnosing Kaposi sarcoma in paraffin-embedded sections. *Am J Clin Pathol.* 2004;121:330–334.
54. McDonagh DP, Liu J, Gaffey MJ, et al. Detection of Kaposi's sarcoma-associated herpesvirus-like DNA sequence in angiosarcoma. *Am J Pathol.* 1996;149:1363–1368.
55. Ahmed I, Hamacher KL. Angiosarcoma in a chronically immunosuppressed renal transplant recipient: report of a case and review of the literature. *Am J Dermatopathol.* 2002;24:330–335.
56. Hammock L, Reisenauer A, Wang W, et al. Latency-associated nuclear antigen expression and human herpesvirus-8 polymerase chain reaction in the evaluation of Kaposi sarcoma and other vascular tumors in HIV-positive patients. *Mod Pathol.* 2005;18:463–468.
57. Pantanowitz L, Pinkus GS, Dezube BJ, et al. HHV8 is not limited to Kaposi's sarcoma. *Mod Pathol.* 2005;18:1148–1150.
58. Alkan S, Eltoum IA, Tabbara S, et al. Usefulness of molecular detection of human herpesvirus-8 in the diagnosis of Kaposi sarcoma by fine-needle aspiration. *Am J Clin Pathol.* 1999;111:91–96.
59. Cobb MW. Human papillomavirus infection. *J Am Acad Dermatol.* 1990;22:547–566.
60. Nuovo GJ, Hochman HA, Eliezri YD, et al. Detection of human papillomavirus DNA in penile lesions histologically negative for condylomata. Analysis by in situ hybridization and the polymerase chain reaction. *Am J Surg Pathol.* 1990;14:829–836.
61. Egawa K. New types of human papillomaviruses and intracytoplasmic inclusion bodies: a classification of inclusion warts according to clinical features, histology and associated HPV types. *Br J Dermatol.* 1994;130:158–166.
62. Kashima M, Adachi M, Honda M, et al. A case of peculiar plantar warts. Human papillomavirus type 60 infection. *Arch Dermatol.* 1994;130:1418–1420.
63. Eng AM, Jin Y-T, Matsuoka LY, et al. Correlative studies of verruca vulgaris by H&E, PAP immunostaining, and electronmicroscopy. *J Cutan Pathol.* 1985;12:46–54.
64. Gross G, Ikenberg H, Gissmann L, et al. Papillomavirus infection of the anogenital region: correlation between histology, clinical picture, and virus type. Proposal of a new nomenclature. *J Invest Dermatol.* 1985;85:147–152.
65. Vogel LN. Epidemiology of human papilloma virus infection. *Semin Dermatol.* 1992;11:226–228.
66. Brown TJ, Yen-Moore A, Tyring SK. An overview of sexually transmitted diseases. Part II. *J Am Acad Dermatol.* 1999;41:661–677.
67. Majewski S, Jablonska S. Human papillomavirus-associated tumors of the skin and mucosa. *J Am Acad Dermatol.* 1997;36:659–685.
68. Gross G, Pfister H, Hagedorn M, et al. Correlation between human papillomavirus (HPV) type and histology of warts. *J Invest Dermatol.* 1982;78:160–164.
69. Jablonska S, Orth G, Obalek S, et al. Cutaneous warts. Clinical, histologic, and virologic correlations. *Clin Dermatol.* 1985;3:71–82.
70. Doorbar J. Papillomavirus life cycle organization and biomarker selection. *Dis Markers.* 2007;23:297–313.
71. Yemelyanova A, Gravitt PE, Ronnett BM, et al. Immunohistochemical detection of human papillomavirus capsid proteins L1 and L2 in squamous intraepithelial lesions: potential utility in diagnosis and management. *Mod Pathol.* 2013;26:268–274.
72. Wititsuwannakul J, Klump VR Jr, McNiff JM, et al. Detecting HPV in cutaneous lesions using anti-HPV antibody immunohistochemistry. *Am J Dermatopathol.* 2013;35:327–331.

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CME Examination Part I

1. In cutaneous infections due to Herpes simplex virus, the strongest immunohistochemical staining is seen in the:

   a. Lymphocytes of the inflammatory infiltrate
   b. Apocrine glands epithelium
   c. Eccrine glands epithelium
   d. Outer root sheath of the hair follicle
   e. Epidermal keratinocytes

2. In cutaneous early infections due to Varicella-zoster virus, the strongest immunohistochemical staining is seen in the:

   a. Outer root sheath of the hair follicle
   b. Sebocytes of the sebaceous gland
   c. Epidermal keratinocytes
   d. Nerves of the dermis
   e. a and b

3. Epstein–Barr virus has been related to all the following diseases, except:

   a. Infectious mononucleosis
   b. Hairy leukoplakia in patients with AIDS
   c. Nasopharyngeal lymphoepithelial carcinoma
   d. Lymphoproliferative disorders
   e. Epidermodysplasia verruciformis

4. In Kaposi's sarcoma, with which of the following monoclonal antibodies will most lesional cells be immunoreactive?

   a. LNA-1
   b. VZV
   c. CM5E1
   d. Ab3
   e. LMP1

5. In which of the following viral-related skin diseases are the histopathological findings not specific?

   a. Trichodysplasia spinulosa
   b. Kaposi's sarcoma
   c. Exanthema subitum
   d. Orf
   e. Merkel cell carcinoma

6. Which of the following HPV-immunohistochemical staining patterns has been suggested as a feature indicating progression in the field of cervical cancer?

   a. Positive HPV-cytoplasmic immunoexpression
   b. Lack of expression of major capsid protein L1
   c. Lack of expression of major capsid protein L2
   d. Expression of the minor capsid protein L2
   e. Lack of expression of major and minor capsid proteins

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ARTICLE 2

Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections - Part II. Cutaneous Viral Infections by Parvoviruses, Polyomaviruses, Poxviruses, Paramyxoviridade, Picornaviridae, Retroviruses and Filoviruses

Immunohistochemistry in the Diagnosis of Cutaneous Viral Infections- Part II: Cutaneous Viral Infections by Parvoviruses, Poxviruses, Paramyxoviridae, Picornaviridae, Retroviruses and Filoviruses

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Background: Cutaneous viral infections are increasing in recent years, particularly in immunocompromised patients.

Objective: Immunohistochemistry (IHC) provides a rapid and helpful tool that can be applied to confirm the diagnosis of specific viral infections that may be difficult to diagnose with certainty using routine microscopy alone.

Methods: Several immunostains that are useful in histopathology have been reviewed and tested in cutaneous samples of viral infections. Emphasis is placed on new stains and novel uses of existing stains.

Results: This article is an up-to-date overview of the potential uses of IHC in the histopathologic diagnosis of cutaneous viral infections by parvoviruses, polyomaviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses, and filoviruses.

Limitations: Specific monoclonal antibodies are commercially available only for some members of these virus families.

Conclusions: IHC may assist dermatopathologists to appropriately diagnose viral infections by parvoviruses, polyomaviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses, and filoviruses.

Key Words: immunohistochemistry, virus, skin, infection, parvoviruses, polyomaviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses, filoviruses

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INTRODUCTION

In this part II of this review, we highlight the available main immunohistochemical techniques that have been used and continue to evolve in the diagnosis of mucocutaneous infections caused by parvoviruses, polyomaviruses, poxviruses, paramyxoviridae, picornaviridae, retroviruses, and filoviruses.

MATERIALS AND METHODS

The biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. For routine histology, 5-μm-thick sections were stained with hematoxylin and eosin. Immunohistochemical stains were performed both at the Pathology Department of Fundación Jiménez Díaz University Hospital in Madrid and the Dermatopathology Laboratory at Friedrichshafen, Germany. Tissue sections were processed on a BioTek Solutions TechMate 500 (Dako), where 1-hour incubation with a primary antibody was performed. The immunohistochemical study of the viral pathogens was performed using the commercially available antibodies. Several antibodies for specific viruses described in the literature were not commercially available at the time of elaboration of this manuscript; therefore, immunohistochemical staining for these pathogens could not be tested.

PARVOVIRUSES

The Parvoviruses are single-stranded DNA viruses and among the smallest known DNA-containing viruses to infect mammalian cells. Parvovirus B19 (PVB19), which belongs to the genus *Erythrovirus*, is the only known human pathogen in this family.[1] Primary infection with PVB19 can be either asymptomatic or bring about a number of clinical syndromes. These include 2 specific PVB19-related dermatologic diseases, erythema infectiosum (fifth disease), and purpuric "gloves-and-socks" syndrome (PPGSS). The most frequently observed dermatological manifestation of PVB19 infection is erythema infectiosum, while PPGSS is rare, and less than 100 cases have been reported.[2] The histopathologic findings in infectious erythema and PPGSS are nonspecific, and usually consist of foci of epidermal spongiosis, with a superficial perivascular infiltrate mostly composed of lymphocytes and some extravasated red cells.[2]

Demonstration of viral DNA in serum and/or skin biopsy samples has been carried out in a handful of reports as a means of linking PPGSS to PVB19 infection.[3–5] However, it has

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become clear that PVB19 can persist for a long time in human tissues,[6] and viral DNA has been found in normal skin with a frequency ranging from 22.5% to 76%.[7,8] This raises the issue whether PVB19 should be regarded as a true pathogen or as an innocent bystander. However, PVB19 can be detected immunohistochemically in the cytoplasm of endothelial cells of congested capillaries in the papillary dermis in skin lesions in PPGSS (Fig. 1) and infectious erythema using the anti-PVB19 monoclonal antibody directed against the viral protein VP2.[2,9,10] Therefore, immunohistochemical detection of PVB19 in the endothelial cells of biopsy specimens performed in the clinical setting of PPGSS and other related exanthemas could be pathogenetically significant and help in the understanding of these diseases.

PVB19 infects cells possessing the receptor for viral entry, the P blood group antigen globoside, with help from coreceptors alpha5beta1 integrin and Ku80.[11] The distribution of viral receptors in different tissues (erythroid progenitors, endothelium, megakaryocytes, fetal myocytes, liver, lung, kidney, synovium, and placental trophoblast) plays a significant role in the pathogenesis of the most frequent syndromes related to PVB19 infection, which include transient aplastic crises, anemia, fetal hydrops, fifth disease, PPGSS, and arthropathy. Santonja et al[2] demonstrated PVB19 endothelial positivity in several cases of PPGSS and proposed that the immunohistochemical detection of PVB19 in the endothelial cells of PPGSS suggests that PVB19 triggers an immunologic response responsible for the histopathologic findings of perivascular lymphocytic inflammation/lymphocytic vasculitis, edema, and red blood cell extravasation.[2] Moreover, involvement of the endothelium by PVB19 is in keeping with the expression of the viral receptor globoside by endothelial cells and suggests that the exanthema results from a cytotoxic T-cell reaction to virally infected cells. Other authors[9,10] have found PVB19 positive labeling not only of the endothelial cells of the dermal vessels but also of the epithelium of eccrine glands, ducts, and epidermal keratinocytes in biopsy specimens of PPGSS.

Endothelial cell involvement by the virus has also been investigated by PVB19 RNA expression in endothelium, by reverse transcriptase in situ polymerase chain reaction (PCR) and by immunohistochemistry (IHC) in the context of vasculitis and autoimmune diseases.[12] The subsequent immunologic reaction has been linked not only with dermatologic conditions (including scleroderma, adult Schönlein purpura, and dermatomyositis)[13–15] but also with systemic diseases, such as systemic lupus erythematosus, myocarditis, and pulmonary fibrosis.[16–18]

POLYOMAVIRUSES

Polyomavirus (PyV) infections were accidentally discovered in the 1950s when characterizing a transmissible agent causing multiple tumors in rodents, hence providing the name (Greek poly-multiple; -oma, tumors).[19] Today the expanding family of human PyV (HPyV) consists of 10 members, but only 2 of them are important for dermatopathologists: the recently identified Merkel cell polyomavirus (MCPyV) and trichodysplasia spinulosa polyomavirus (TSPyV). Serological studies suggest that HPyVs subclinically infect the general population with rates ranging from 35% to 90%. However, significant disease is only observed in patients with impaired immune functions.[20]

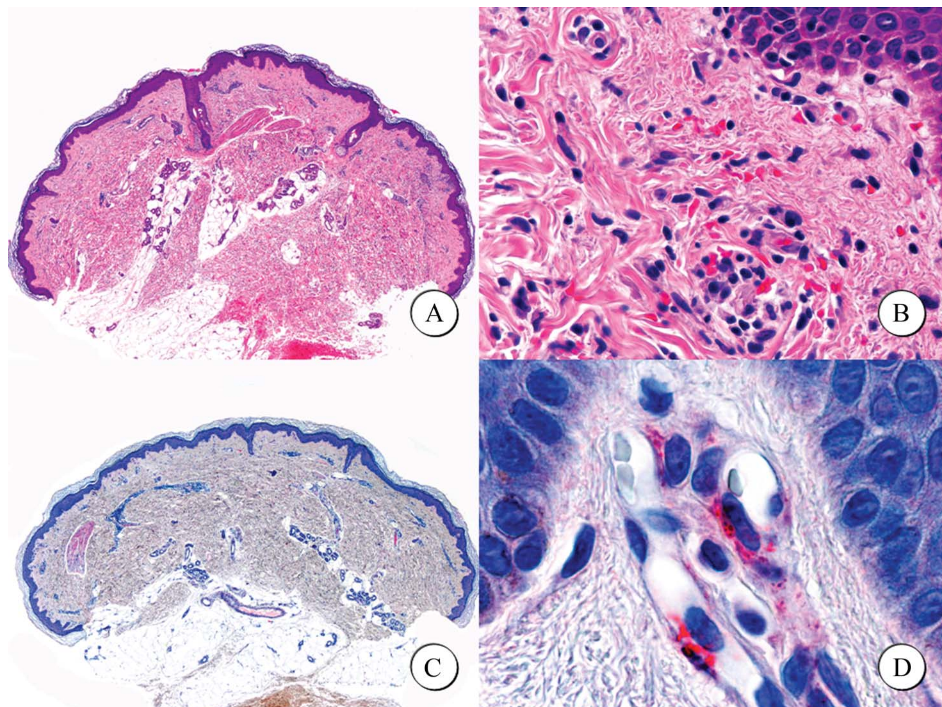

FIGURE 1. Histopathologic and immunohistochemical findings in the cutaneous lesions of a patient with PPGSS. A, Scanning power showing discrete perivascular infiltrates in the superficial dermis. B, Higher magnification demonstrated perivascular lymphocytes and extravasated red cells. C, The same case immunohistochemically studied with the anti-PVB19 monoclonal antibody directed against the viral protein VP2. D, Immunoexpression for PVB19 within the cytoplasm of endothelial cells (A and B, hematoxylin–eosin stain; C and D, IHC for PVB19 antibody; original magnifications: A ×10, B ×400, C ×10, and D ×400).

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Merkel Cell Polyomavirus

Merkel cell carcinoma (MCC) is a rare but aggressive human skin cancer that typically affects elderly and immunosuppressed individuals, a feature suggestive of an infectious origin. The search for an infectious agent yielded a major breakthrough in 2008; the discovery of the MCPyV by means of digital transcriptome subtraction.[21] Numerous studies rapidly validated the association of MCC with this virus that is integrated in the nuclei of MCC neoplastic cells.[22–24] Although the association between MCC and the MCPyV is now well established, it is also clear that this virus is not required for MCC as approximately 20% of these tumors contain no detectable MCPyV. Moreover, it is clear that MCPyV is not sufficient for developing MCC because it remains a rare cancer despite the fact that more than half of adults have antibodies to MCPyV and hence were exposed to the virus, typically in childhood.[24,25] Finally, MCPyV is not exclusive to MCC because it has also been observed rarely in certain squamous cell carcinomas in immunosuppressed patients.[26,27]

Since the original report, multiple studies have demonstrated the clonal integration of MCPyV in the nucleus of MCC cells in approximately 80% of the cases. A variety of methods have been used to detect the presence of the virus, including Southern blot analysis, PCR amplification of viral DNA, detection of integrated PyV sequences, in situ hybridization, DNA sequencing by hybrid capture, RT-PCR, and IHC with antibodies specific for MCPyV large T antigen and small T antigen.[24,28–30] Based on homology to other polyomaviruses, the MCPyV large and small T antigens are predicted to be oncogenic and contribute directly to the carcinogenesis of MCC.[30]

Shuda et al[29] developed a monoclonal antibody CM2B4 against a peptide fragment of the MCPyV T antigen exon 2 to detect MCPyV oncoprotein expression in the MCC tumor cells directly. The majority of the CM2B4-positive tumors showed strong and diffuse nuclear labeling in a large part of the tumor cell population (Fig. 2) and 77% of the PCR-positive tumors were immunoreactive for CM2B4. Later, these same authors[31] reported that the CM2B4 antibody failed to detect large T antigen in several cases of MCC but that many of these negative specimens stained positive with the monoclonal antibody CM5E1 specific for MCPyV small T antigen. The combined use of CM2B4 and CM5E1 led to detection of MCPyV T antigens in 47 (92%) of 51 cases of MCC, whereas CM2B4 alone detected large T antigen in 75% of cases.

Recently, Rodig et al[30] have performed IHC staining with a newly developed mouse monoclonal antibody, Ab3, that has markedly increased sensitivity in detecting MCPyV large T antigen (97%) in MCC compared with CM2B4 (80%). They propose that the presence of MCPyV in MCC is more common than previously reported and that improved detection methods may reveal that practically all MCC specimens contain viral DNA of MCPyV. Ab3 does not stain lymphocytes, even when used at higher concentrations or when staining tonsillar and reactive lymph node tissue specimens. The lack of nonspecific staining may be especially useful in clinical specimens because MCC tumors frequently contain infiltrating lymphocytes.[32] Furthermore, Ab3 did not stain tumor specimens from gastrointestinal neuroendocrine tumors or small cell lung cancer, supporting its usefulness as a clinical biomarker for MCC.

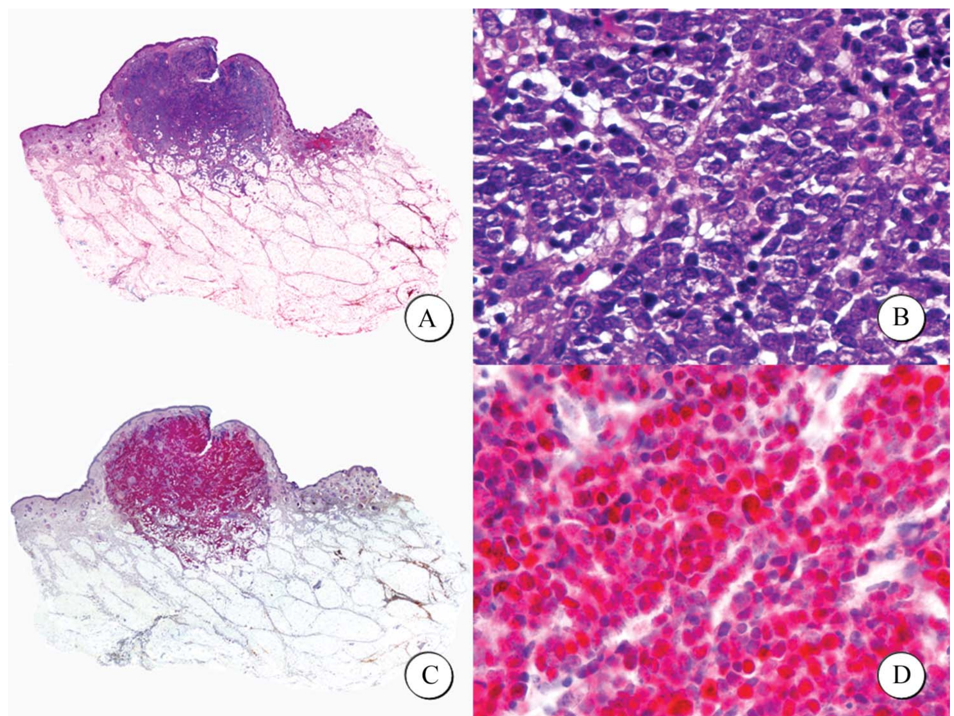

FIGURE 2. Histopathologic and immunohistochemical findings in a MCC. A, Scanning power showing a neoplasm that involved the full thickness of the dermis and extended to subcutaneous tissue. B, Higher magnification demonstrated that the tumor was composed of small round cells with vesicular nuclei and multiple small nucleoli. C, The same case immunohistochemically studied with the monoclonal antibody CM2B4 against a peptide fragment of the MCPyV. D, Immunoexpression for CM2B4 is seen in the nuclei of neoplastic cells (A and B, hematoxylin–eosin stain; C and D, IHC for CM2B4 antibody; original magnifications: A ×10, B ×400, C ×10, and D ×400).

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Several studies[26,27] have shown that MCPyV DNA may be identified by PCR in up to 70% of nonmelanocytic cutaneous neoplasms in immunocompetent and immunosuppressed patients. Unlike MCC, these skin tumors exhibit no immunohistochemical evidence for intracellular viral antigens. Inflammatory cells may have transported viral DNA into peritumoral infiltrates as suggested by the fact that inflammatory $CD14^{+}$ $CD16^{-}$ monocytes constitute a reservoir for MCPyV.[33] When interpreting MCPyV detection, it is therefore essential to consider both molecular biology and immunohistochemical results.

Trichodysplasia Spinulosa Polyomavirus

Trichodysplasia spinulosa (TS) is a folliculocentric skin disorder that presents as keratotic spiny papules typically distributed over the ears and structures of the central face and less commonly involves the extremities, trunk, and scalp. Alopecia, usually most severely affecting the eyebrows and eyelashes, often accompanies the disorder. Haycox et al[34], in 1999, were the first authors using the term TS to describe friable follicular spinous lesions in a patient who received a combined renal/pancreas transplant. The authors showed, for the first time, the intracellular presence of virus particles with an appearance that was interpreted by them as consistent with viral particles of the Papovaviridae family. However, the original description of this disorder was published by Izakovic et al, in 1995, when they reported hair-like hyperkeratosis in patients with kidney transplants and considered the process as a new cyclosporine side-effect.[35] Since then, several cases have been reported in the literature[20,36–67] under different names, including viral-associated trichodysplasia,[39,43,49,52,60] trichodysplasia of immunosuppression,[45] virus-associated TS,[40] follicular dystrophy of immunosuppression,[37] cyclosporine-induced folliculodystrophy,[38] pilomatrix dysplasia,[36] and spiny hyperkeratosis (hair-like hyperkeratosis).[41] In 2010, van der Meijden et al[46] identified a new HPyV (TSPyV) in plucked facial spines of a heart transplant patient with TS, confirming the PyV etiology of this process.

The histopathologic findings are distinctive and consist of distorted and dilated anagen hair follicles filled by sheets of eosinophilic cells exhibiting the appearance of inner root sheath cells.[34,35] These cells, which contain abnormally large trichohyaline granules, abruptly cornify without the presence of a granular cell layer. The involved follicles, instead of the full keratinization toward a mature hair shaft, appear replete with sheets of nucleated cornifying cells that persist throughout most of the lower segment of the hair follicles. The observation was made that pathogenesis seemed to be related to immunosuppression, with the lesions resolving as immune function returned to normal.

Several tools, including scanning electron microscopy, PCR analysis of viral DNA, or immunohistochemical staining for the PyV middle T antigen, have been used to identify the pathogenic virus associated with TS. In the initial description of viral-associated TS, immunohistochemical stains demonstrated increased Ki-67 protein expression and negative staining for Papillomavirus.[34] At that time, the initial virus was assumed to be part of the Papovaviridae family, which has subsequently been split into the Papillomaviridae and Polyomaviridae families. Most studies in TS have identified TSPyV by electron microscopy,[39,40,42,43,49,54,55,57] PCR studies,[20,49,54,55,57,59,66] or immunofluorescence,[54] but very few attempts have been performed by IHC.[55,60] In the case described by Fischer et al,[55] immunohistochemical investigation for the HPyV middle T antigen with the SV40 antibody, which recognized the NH2 terminus within amino acids 83–128 of a large tumor antigen of SV-40 and JCV of PyVs, failed to detect the virus in lesional skin, renal allograft, and urine specimens.[55] However, Wanat et al[60] have recently described immunohistochemical positivity with SV-40 antibody for TSPyV in lesional skin of a patient with TS, demonstrating positive staining of the large eosinophilic cellular inclusions within keratinocytes composing the inner root sheath in all the follicles examined in the patient's samples, and we have corroborated these findings (Fig. 3). These positive cellular inclusions are visualized on both vertical and horizontal sections, and scanning electron microscopy confirmed that these inclusions contained small, icosahedral, regularly spaced, intracellular viral particles consistent with PyV.[60] These authors support that immunohistochemical staining for the middle T antigen or other viral proteins may be a useful tool for the identification of this disease process and a potentially more useful and practical way to establish the diagnosis compared with PCR assay or electron microscopy.[60] However, further cases are required to verify the utility of immunohistochemical staining because a negative result would not exclude the diagnosis.

Finally, routine testing with PCR assay, electron microscopy, or immunohistochemical stains is not necessary in all cases because routine histologic staining can be sufficient to make the diagnosis in cases of classic clinical presentations. Because the true incidence and clinical spectrum of this recently recognized disease is not known, these tools may be valuable in confirming the diagnosis in cases involving more subtle or atypical presentations. It is important that clinicians are aware of all the potential evaluations that can be performed and that they recognize the newly described HPyV as the infectious agent that is responsible for TS.

POXVIRUSES

The family Poxviridae is divided into many genera, of which the genus *Orthopoxvirus* includes vaccinia virus, variola virus, cowpox virus, and at least 6 other species, including monkeypox virus, camelpox virus, and raccoonpox virus.[68,69] Only 3 other genera cause human disease: the genus *Parapoxvirus* causing milker's nodule, orf, and sealpox[69–71]; the genus/subgenus *Molluscipoxvirus* resulting in molluscum contagiosum[72]; and the genus *Yatapoxvirus* resulting in tanapox. Skin infections caused by parapoxvirus have characteristic histopathology.

Orf (Ecthyma Contagiosum)

Orf (ecthyma contagiosum) is primarily a disease of young sheep and goats, involving the lips and perioral area.[1] It is caused by a poxvirus of the paravaccinia subgroup. Orf can be transmitted to humans by contact with infected animals. Lesions, which measure approximately 1–3 cm or more in

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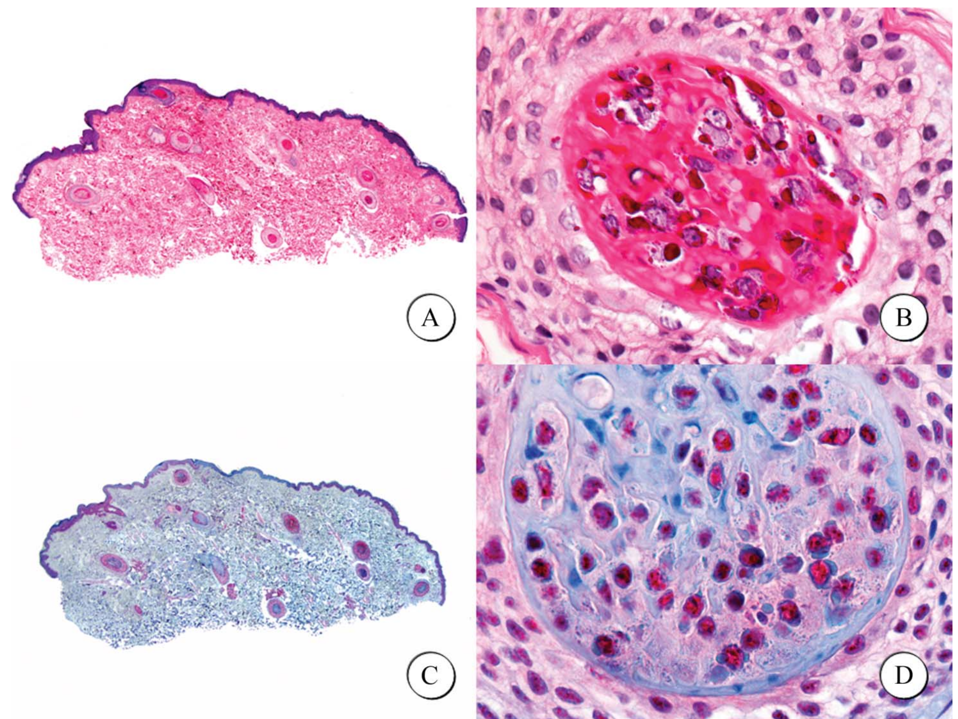

FIGURE 3. Histopathologic and immunohistochemical findings in cutaneous lesions of a patient with TS. A, Scanning power showing involvement of all hair follicles present in the biopsy. B, Higher magnification demonstrated that the inner root sheath cells contained abnormally large trichohyaline granules. C, The same case immunohistochemically studied with the monoclonal antibody SV-40 for TSPyV. D, Immunoexpression for SV-40 is seen in the large trichohyaline granules of the inner root sheath cells (A and B, hematoxylin–eosin stain; C and D, IHC for SV-40 antibody; original magnifications: A ×10, B ×400, C ×10, and D ×400).

diameter, develop most commonly on the hands and forearms. Spontaneous regression is usual approximately in 7–8 weeks.[1]

The diagnosis is based on case history (contact with infected animals) and clinical features.[73] Viral cultures, histopathologic examination of biopsy specimens, electron microscopy, fluorescent antibody tests, and identification of specific viral nucleic acid have all contributed to establish the diagnosis.[73–76] Orf has a distinctive histological appearance of hyperkeratosis and cell ballooning in the cells of the upper layers of the epidermis. Eosinophilic intracytoplasmic inclusions are seen within these ballooned cells. The lesions of orf are generally regarded as histopathologically indistinguishable from milker's nodules,[77] although full-thickness epidermal necrosis seems to be more common in orf. Recently, a monoclonal antibody against the ORFV059 protein encoded by orf virus have been synthesized, which recognized the ORFV-Jilin ORFV059 protein in a variety of immunological assays, but to our knowledge, this antibody is not yet commercially available.[78]

PARAMYXOVIRIDAE

Measles

Measles is a highly contagious viral disease caused by an RNA virus of the Paramyxoviridae family, and human beings are the natural host and reservoir of infection. It spreads through the respiratory route and the virus tends to involve the lymph nodes, followed by viremia and infection of any organ, including the skin.[79] The disease is characterized by fever, malaise, cough, nasal congestion, conjunctivitis, and an erythematous maculopapular skin rash that appears 3 or 4 days after the onset of fever and spreads in a craniocaudal direction becoming confluent as the disease progresses. A pathognomonic enantem, named Koplik spots, appears during prodrome and consists of white papules on the oral mucosa. The disease is largely self-limiting in immunocompetent and immunized individuals but may be severe and even deadly in immunocompromised patients. All countries in the World Health Organization have renewed their commitment to eliminate measles transmission by 2015. However, measles has re-emerged in Europe recently because of suboptimal immunization levels that led to accumulation of susceptible populations over the last years.[80]

From an histopathologic point of view, the findings in skin rash of measles are usually nonspecific. Examination of the biopsy specimen usually shows basal vacuolar degeneration and mild dermal lymphocytic infiltrate, which usually is predominantly perifollicular. In some cases, multiple necrotic keratinocytes in the superficial epidermis, multinucleated intraepidermal cells, and pyknotic keratinocytes in the hair follicle epithelium have been described.[81,82] In the skin and oral mucous lesions (Koplik's spots), multinucleated keratinocytes with pyknotic nuclei have reported in the upper layers of the epithelium,[82,83] in the hair follicles,[84] and in the acrosyringium,[85] and they have been proposed as a cytopathologic clue for specific diagnosis. Intranuclear and intracytoplasmic inclusion bodies and abundant eosinophils in the infiltrate have been also described.[86]

Electron microscopic, immunofluorescence, and immunohistochemical studies have detected the measles virus in lesional skin within endothelial cells of dermal capillaries,[87] epidermal multinucleate cells,[83] dermal fibroblasts and macrophages,[87] lymphocytes,[84] keratinocytes of the upper layers of the epidermis,[84,88,89] hair follicles,[84,88,90] and sebaceous glands.[88] IHC detects the measles virus–specific antigens with a commercially available monoclonal antibody directed

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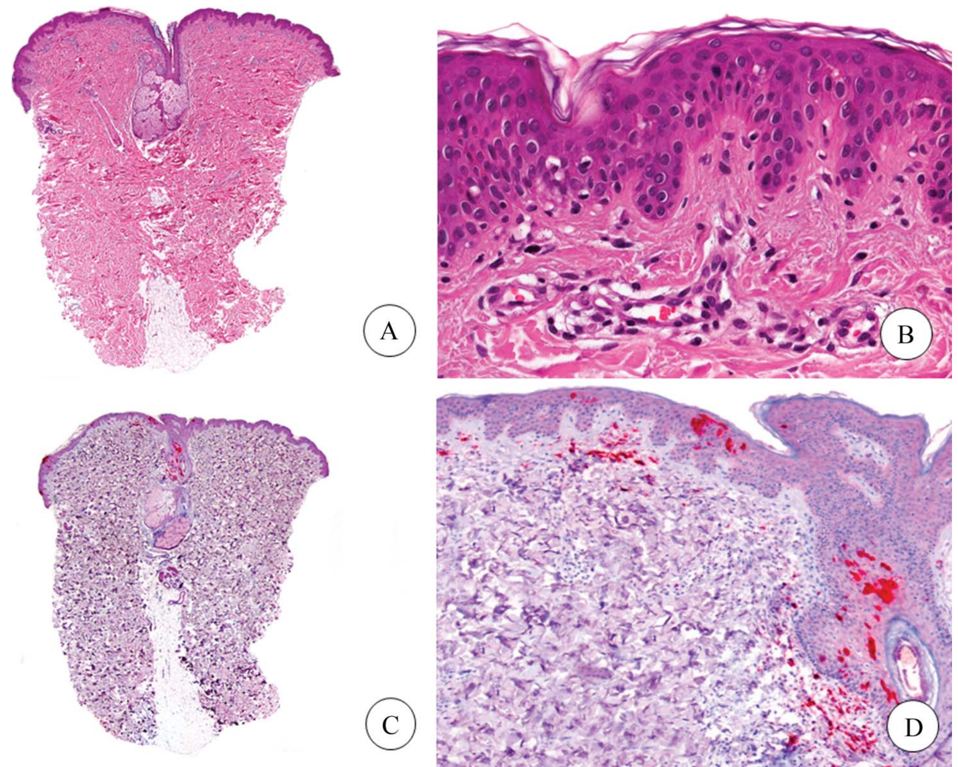

FIGURE 4. Histopathologic and immunohistochemical findings in the exanthema of patient with measles. A, Scanning power showing apparently normal skin. B, Higher magnification demonstrated isolated necrotic keratinocytes in the epidermis and mild superficial perivascular lymphocytic infiltrate. C, The same case immunohistochemically studied with the antibody for measles virus. D, Immunoexpression is seen in the nuclei of epidermal keratinocytes, keratinocytes of the hair follicle, and lymphocytes of the dermal infiltrate (A and B, hematoxylin–eosin stain; C and D, IHC for measles antibody; original magnifications: A ×10, B ×400, C ×10, and D ×200).

against the viral nucleoprotein[90] (Fig. 4). It has also been demonstrated that wild-type strains of measles virus use the signaling lymphocyte activation molecule (SLAM, also named CDw150) as a cellular receptor. In contrast, laboratory strains of measles virus and its derivate vaccine strains use both SLAM and the complement regulatory protein CD46 as cellular receptors.[91] Immunohistochemical studies of the skin of measles patients using monoclonal antibodies anti-SLAM and anti-CD46 have shown that hair follicles, epidermis, capillary blood vessels, fibroblasts, mononuclear cells, and acrosyringium were CD46 positive and SLAM negative.[90,92] These results suggest that CD46 may be a receptor of the wild measles virus in the skin.[90,93]

PICORNAVIRIADE

Enteroviruses

Enteroviruses comprise a subgroup of *Picornaviridae* family that cause a wide spectrum of disorders associated with exanthemas. The non-polio enteroviruses include echoviruses and coxsackie types A and B. These viruses have a single-stranded RNA genome and an unenveloped capsid.

Enteroviral infections are worldwide infections transmitted by fecal–oral or respiratory route. Enteroviruses first infect epithelial cells of the upper airway or lower alimentary tract, replicate in lymph nodes, and disseminate by an initial viremia. Subsequently, replication occurs in many organs and during this replication period is when clinical manifestations appear. A second and major viremia occurs during this period or viral replication occurs in the secondary infection sites.[94]

Hand-foot-and-mouth disease (HFMD) is the most characteristic exanthematous disease caused by enteroviruses. Numerous coxsackie serotypes have been implicated, coxsackie A16 being the most common etiologic factor. Prodromos consist of fever and malaise and few days later the exanthema appears. Dermatologic manifestations are characterized by the abrupt onset of elongated ovoid small vesicles on the palms and soles, in conjunction with erosive stomatitis. In rare cases, vesicles may also involve dorsum of the hands and feet and other nonacral areas of the skin. Most patients with HFMD have a benign and self-limited course, although in rare cases of outbreaks of severe enterovirus 71 infection, cardiopulmonary and neurologic complications and even death of involved patients have been described.[95]

Herpangina is a pediatric febrile disease cause by coxsackie virus groups A and B or echoviruses characterized by painful vesicles and erosions in buccal mucosa, soft palate, and tonsils.[96] Enteroviruses may also be the etiologic agent for the so-called eruptive pseudoangiomatosis, which consists of spontaneously regressing vascular papules composed of telangiectatic vessels, but without a proliferative component.[97,98]

Histopathologically, HFMD lesions show intraepidermal vesicles with prominent reticular degeneration and a few ballooned cells. No multinucleate cells or inclusion bodies have been described in lesions of HFMD. Usually, there is accompanying papillary dermal edema and a mild perivascular inflammatory infiltrate mostly composed of lymphocytes. Electron microscopic studies demonstrate viral particles.[99] From an immunohistochemical point of view, there is a commercially available antibody that reacts with an epitope on the VP1 peptide, which is highly conserved within the enterovirus group.[100]

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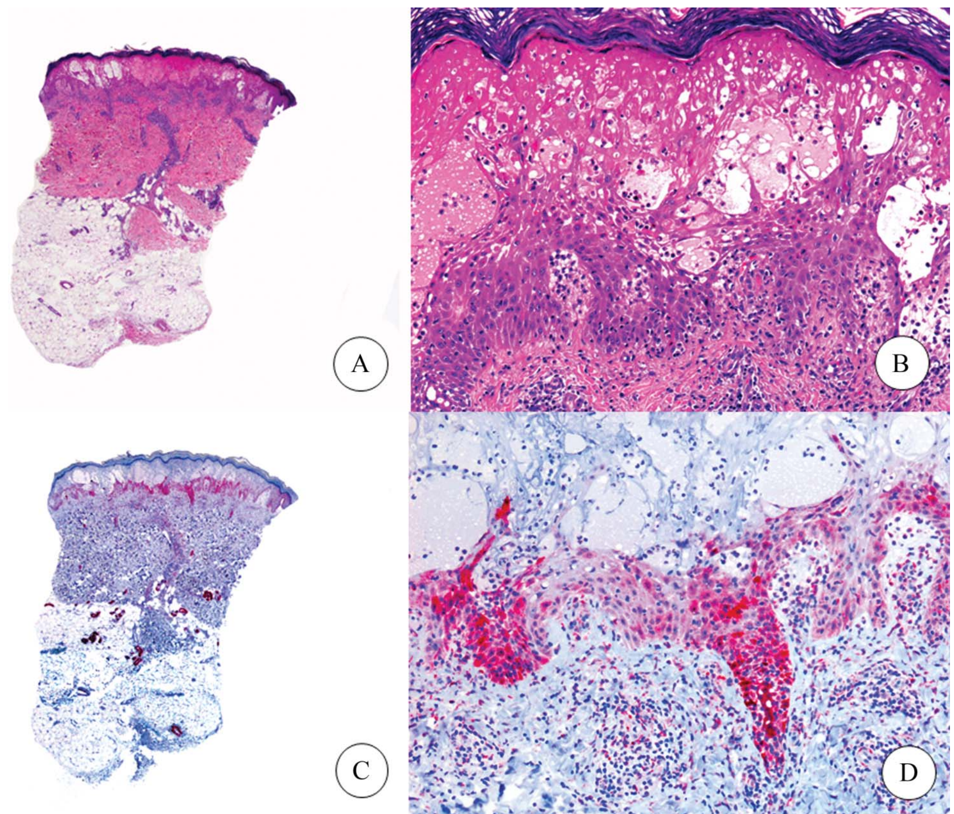

FIGURE 5. Histopathologic and immunohistochemical findings in cutaneous lesions of a patient with HFMD. A, Scanning power showing severe epidermal involvement. B, Higher magnification demonstrated intraepidermal vesicle with prominent reticular degeneration and a few ballooned cells. C, The same case immunohistochemically studied with the monoclonal antibody that reacts with the VP1 peptide of the enterovirus group. D, Immunoexpression for VP1 peptide of the enterovirus group is seen in epidermal keratinocytes and acrosyringeal keratinocytes (A and B, hematoxylin–eosin stain; C and D, IHC for enterovirus antibody; original magnifications: A ×10, B ×40, C ×10, and D ×200).

This antibody reacts with a molecule of 34–37 kDa molecular weight, and it was generated by using coxsackie B5 as immunogen. It reacts with most enteroviruses of echovirus, coxsackie and poliovirus group, but does not react with rotavirus, yellow fever virus, measles, rhinovirus A1, adenovirus 18, or hepatitis A virus.[101] In our experience, this generic anti-enterovirus antibody stains mostly keratinocytes of the acrosyringium in lesional skin (Fig. 5).

RETROVIRUSES

Human T-Cell Lymphotropic Virus Type 1

Human T-cell lymphotropic virus type 1 (HTLV-1), also named human T-cell leukemia virus type 1 and adult T-cell lymphoma virus type 1, is endemic in the south of Japan and in the Caribbean Islands and rare in other regions. Transmission of HTLV-1 occurs primarily by sexual intercourse, blood transfusions, needle sharing among intravenous drug abusers, and breastfeeding. HTLV-1 induces T-cell proliferation, followed by interleukin (IL)-2 receptor exposition, increased IL-2 secretion, and induction of interferon-γ, IL-5, and IL-10.

HTLV-1 is associated with infective dermatitis and adult T-cell leukemia/lymphoma (ATLL). Infective dermatitis is an exudative and eczematous dermatosis that involves mostly the scalp, axillae, groin, and small folds of the face.[102] In some cases, infective dermatitis may progress to more severe HTLV-1–associated disease, such as ATLL. Four variants of ATLL are recognized in the new World Health Organization classification of tumors of hematopoietic and lymphoid tissues[103]: acute and chronic leukemic, lymphomatous, and smoldering types. Although cutaneous manifestations are usually seen in the smoldering form of the disease, it has been suggested that patients with purely cutaneous lesions may have a better prognosis and should be classified separately from those with smoldering ATLL.[104] Cutaneous manifestations and histopathologic features of ATLL are identical to those of mycosis fungoides, so demonstration of retroviral infection is mandatory for diagnosis. Molecular analyses show a monoclonal rearrangement of the TCR gene and the presence of the integrated genome of HTLV-I.[105] In the early phase of the ATLL, neoplastic cell properties dependent on the HTLV-1 proviral DNA pX region p40Tax protein (Tax)[101–109] appear under the effects of external and internal mutagens[110–112] following the accumulation of mutations induced by repeated Tax expression[113] in the background of persistent proliferation of HTLV-1–infected T cells, which is probably induced by HTLV-1 basic leucine zipper (HBZ) messenger RNA.[114,115] Then, late-phase ATLL cells with neoplastic properties independent of Tax appear when mutation has progressed in the host cell DNA and HTLV-1 proviral DNA, including pX p40Tax DNA sequence.[110] Histopathological diagnosis of ATLL is expected based on IHC detecting of Tax, HTLV-1 proviral DNA pX p27Rex protein (Rex), or HBZ messenger RNA/protein in neoplastic cells[116] (Fig. 6).

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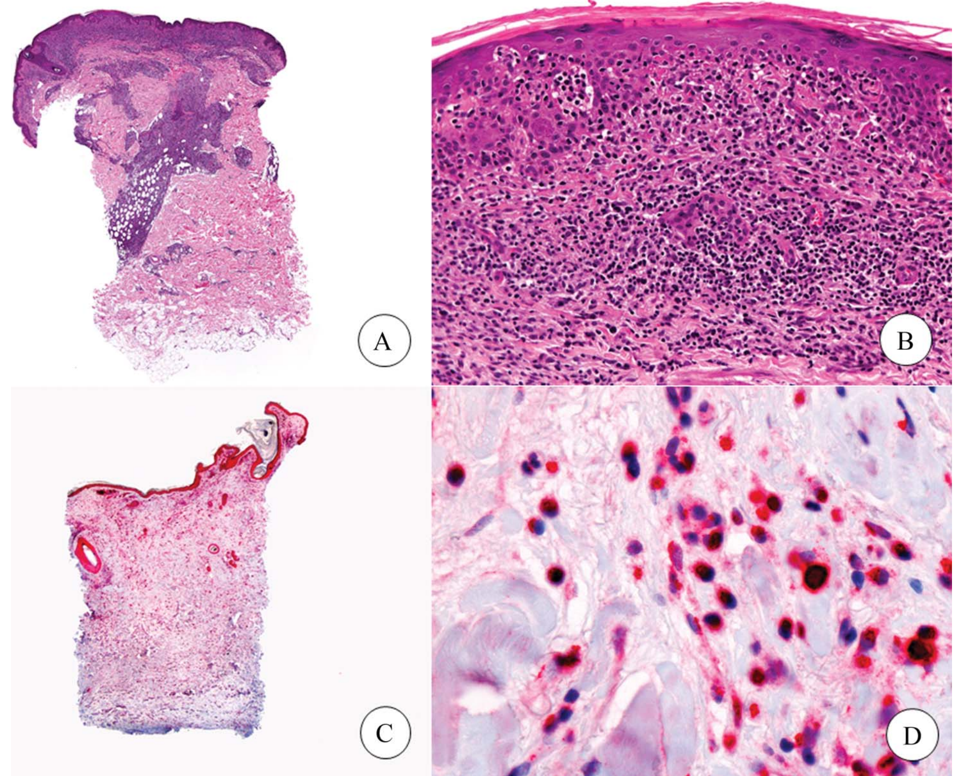

FIGURE 6. Histopathologic and immunohistochemical findings in cutaneous lesions of a patient with ATLL. A, Scanning power showing a dense band-like infiltrate in the superficial dermis and dense perivascular and perifollicular infiltrate. B, Higher magnification demonstrated the epidermotropism of the infiltrate in some areas. C, The same case immunohistochemically studied with the monoclonal antibody against HTLV-1. D, Immunoexpression for HTLV-1 is seen as a dot-like paranuclear positivity, probably located in Golgi apparatus of neoplastic lymphocytes. Large positive cells are mast cells expressing nonspecific immunostaining (A and B, hematoxylin–eosin stain; C and D, IHC for HTLV-1; original magnifications: A ×10, B ×40, C ×10, and D ×400).

FILOVIRUSES

The family Filoviridae is an unique negative-stranded RNA virus family that forms filamentous virions. Not much is known about filoviruses because their highly pathogenic nature makes them difficult to study. Two members of the family that are commonly known are Ebola virus and Marburg virus.[117] Both viruses, and some of their lesser known relatives, cause severe disease in humans and nonhuman primates in the form of viral hemorrhagic fevers.

Ebola Virus

Ebola virus causes a severe and often fatal hemorrhagic fever.[117] Ebola hemorrhagic fever (EHF) is a rare disease, and the number of sporadic cases occurring in tropical Africa is unknown. The clinical diagnosis of EHF is often presumptive, and laboratory confirmation is essential. Traditionally, the laboratory diagnosis of EHF has been accomplished through virus isolation or serologic assays.[118,119] Because of the biosafety hazards associated with the handling and testing of EBO virus, these assays can be performed in only a few specialized laboratories worldwide, requiring transportation of the dangerous biological specimens from remote sites to these laboratories.

In 1999, Zaki et al[120] reported the development of a novel, safe, sensitive, and specific diagnostic immunohistochemical test for Ebola virus infections, which uses formalin-fixed postmortem skin specimens, and should be useful for EHF surveillance and prevention. This antibody is not commercially available. These authors evaluated skin biopsies of 14 patients during the EHF outbreak in the Democratic Republic of Congo in 1995. They found abundant viral antigens and particles within endothelial cells, mononuclear phagocytic cells, and fibroblasts in the skin of EHF patients using IHC, thus suggesting an epidemiologic role for contact transmission. Histopathologic changes in the skin tissue were not pathognomonic and consisted mainly of various degrees of endothelial cell swelling and necrosis.

CONCLUSIONS

IHC is an excellent diagnostic technique with the distinct advantage of being able to exactly locate a given protein within the tissue examined. The field is continuously expanding, with new applications steadily increasing. Ultimately, the possibilities of IHC in the field of cutaneous viral infections are immense, and the future is very promising.

REFERENCES

1. Weedon D. Viral diseases. In: Weedon D, ed. *Weedon's Skin Pathology*. 3rd ed. Philadelphia, PA: Churchil Livingstone Elsevier; 2010:608–631.
2. Santonja C, Nieto-González G, Santos-Briz Á, et al. Immunohistochemical detection of parvovirus B19 in "gloves and socks" papular purpuric syndrome: direct evidence for viral endothelial involvement. Report of three cases and review of the literature. *Am J Dermatopathol.* 2011;33: 790–795.
3. Grilli R, Izquierdo MJ, Fariña MC, et al. Papular-purpuric "gloves and socks" syndrome: polymerase chain reaction demonstration of parvovirus B19 DNA in cutaneous lesions and sera. *J Am Acad Dermatol.* 1999;41:793–796.

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4. Aractingi S, Bakhos D, Flageul B, et al. Immunohistochemical and virological study of skin in the papular-purpuric gloves and socks syndrome. *Br J Dermatol.* 1996;135:599–602.
5. Sklavounou-Andrikopoulou A, Iakovou M, Paikos S, et al. Oral manifestations of papular-purpuric "gloves and socks" syndrome due to parvovirus B19 infection: the first case presented in Greece and review of the literature. *Oral Dis.* 2004;10:118–122.
6. Norja P, Hokynar K, Aaltonen LM, et al. Bioportfolio: lifelong persistence of variant and prototypic erythrovirus DNA genomes in human tissue. *Proc Natl Acad Sci U S A.* 2006;103:7450–7453.
7. Bonvicini F, La Placa M, Manaresi E, et al. Parvovirus B19 DNA is commonly harboured in human skin. *Dermatology*. 2010;220: 138–142.
8. Corcioli F, Zakrzewska K, Rinieri A, et al. Tissue persistence of parvovirus B19 genotypes in asymptomatic persons. *J Med Virol.* 2008;80: 2005–2011.
9. Schwarz TF, Wiersbitzky S, Pambor M. Case report: detection of parvovirus B19 in a skin biopsy of a patient with erythema infectiosum. *J Med Virol.* 1994;43:171–174.
10. Takahashi M, Ito M, Sakamoto F, et al. Human parvovirus B19 infection: immunohistochemical and electron microscopic studies of skin lesions. *J Cutan Pathol.* 1995;22:168–172.
11. Munakata Y, Saito-Ito T, Kumura-Ishii K, et al. Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. *Blood*. 2005; 106:3449–3456.
12. Magro CM, Crowson AN, Dawood M, et al. Parvoviral infection of endothelial cells and its possible role in vasculitis and autoimmune diseases. *J Rheumatol.* 2002;29:1227–1235.
13. Magro CM, Iwenofu OH, Kerns MJ, et al. Fulminant and accelerated presentation of dermatomyositis in two previously healthy young adult males: a potential role for endotheliotropic viral infection. *J Cutan Pathol.* 2009;36:853–858.
14. Cioc AM, Sedmak DD, Nuovo GJ, et al. Parvovirus B19 associated adult Henoch Schönlein purpura. *J Cutan Pathol.* 2002;29:602–607.
15. Magro CM, Nuovo G, Ferri C, et al. Parvoviral infection of endothelial cells and stromal fibroblasts: a possible pathogenetic role in scleroderma. *J Cutan Pathol.* 2004;31:43–50.
16. Hession MT, Au SC, Gottlieb AB. Parvovirus B19-associated systemic lupus erythematosus: clinical mimicry or autoimmune induction? *J Rheumatol.* 2010;37:2430–2432.
17. Bock CT, Klingel K, Kandolf R. Human parvovirus B19-associated myocarditis. *N Engl J Med.* 2010;362:1248–1249.
18. Magro CM, Wusirika R, Frambach GE, et al. Autoimmune-like pulmonary disease in association with parvovirus B19: a clinical, morphologic, and molecular study of 12 cases. *Appl Immunohistochem Mol Morphol.* 2006;14:208–216.
19. Gross L. A filterable agent, recovered from Ak leukemic extracts, causing salivary gland carcinomas in C3H mice. *Proc Soc Exp Biol Med.* 1953;83:414–421.
20. Dalianis T, Hirsch HH. Human polyomaviruses in disease and cancer. *Virology*. 2013;437:63–72.
21. Feng H, Shuda M, Chang Y, et al. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science*. 2008;319:1096–1100.
22. Kassem A, Technau K, Kurz AK, et al. Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. *Int J Cancer.* 2009;125:356–361.
23. Garneski KM, Warcola AH, Feng Q, et al. Merkel cell polyomavirus is more frequently present in north American than Australian Merkel cell carcinoma tumors. *J Invest Dermatol.* 2009;129:246–248.
24. Busam KJ, Jungbluth AA, Rekthman N, et al. Merkel cell polyomavirus expression in Merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. *Am J Surg Pathol.* 2009;33:1378–1385.
25. Carter JJ, Paulson KG, Wipf GC, et al. Association of Merkel cell polyomavirus-specific antibodies with Merkel cell carcinoma. *J Natl Cancer Inst.* 2009;101:1510–1522.
26. Andres C, Belloni B, Puchta U, et al. Prevalence of MCPyV in Merkel cell carcinoma and non-MCC tumors. *J Natl Cancer Inst.* 2009;101: 1655–1666.
27. Mertz KD, Pfaltz M, Junt T, et al. Merkel cell polyomavirus is present in common warts and carcinoma in situ of the skin. *Hum Pathol.* 2010; 41:1369–1379.
28. Jung HS, Choi YL, Choi JS, et al. Detection of Merkel cell polyomavirus in Merkel cell carcinomas and small cell carcinomas by PCR and immunohistochemistry. *Histol Histopathol.* 2011;26:1231–1241.
29. Shuda M, Arora R, Kwun HJ, et al. Human Merkel cell polyomavirus infection I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. *Int J Cancer.* 2009;125: 1243–1249.
30. Rodig SJ, Cheng J, Wardzala J, et al. Improved detection suggests all Merkel cell carcinomas harbor Merkel polyomavirus. *J Clin Invest.* 2012;122:4645–4653.
31. Shuda M, Kwun HJ, Feng H, et al. Human Merkel cell polyomavirus small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. *J Clin Invest.* 2011;121:3623–3634.
32. Paulson KG, Iyer JG, Tegeder AR, et al. Transcriptome-wide studies of Merkel cell carcinoma and validation of intratumoral CD8+ lymphocyte invasion as an independent predictor of survival. *J Clin Oncol.* 2011;29: 1539–1546.
33. Mertz KD, Junt T, Schmid M, et al. Inflammatory monocytes are a reservoir for Merkel cell polyomavirus. *J Invest Dermatol.* 2010;130: 1146–1151.
34. Haycox CL, Kim S, Fleckman P, et al. Trichodysplasia spinulosa—a newly described folliculocentric viral infection in an immunocompromised host. *J Investig Dermatol Symp Proc.* 1999;4:268–271.
35. Izakovic J, Büchner SA, Düggelin M, et al. Haarartige Hyperkeratosen bei einem Nierentransplantierten. Eine neue Cyclosporin-Nebenwirkung. *Hautarzt*. 1995;46:841–846.
36. Chastain MA, Millikan LE. Pilomatrix dysplasia in an immunosuppressed patient. *J Am Acad Dermatol.* 2000;43:118–122.
37. Daneshpazhooh M, Asgari M. Follicular dystrophy of immunosuppression. *J Am Acad Dermatol.* 2005;52(3 pt 1):540; author reply 540–541.
38. Heaphy MR, Shamma HN, Hickmann M, et al. Cyclosporine-induced folliculodystrophy. *J Am Acad Dermatol.* 2004;50:310–315.
39. Sperling LC, Tomaszewski M, Thomas D. Viral-associated trichodysplasia in patients who are immunocompromised. *J Am Acad Dermatol.* 2004;50:318–322.
40. Wyatt AJ, Sachs DL, Shia J, et al. Virus-associated trichodysplasia spinulosa. *Am J Surg Pathol.* 2005;29:241–246.
41. Campbell RM, Ney A, Gohh R, et al. Spiny hyperkeratotic projections on the face and extremities of a kidney transplant recipient. *Arch Dermatol.* 2006;142:1643–1648.
42. Sadler GM, Halbert AR, Smith N, et al. Trichodysplasia spinulosa associated with chemotherapy for acute lymphocytic leukaemia. *Australas J Dermatol.* 2007;48:110–114.
43. Osswald SS, Kulick KB, Tomaszewski MM, et al. Viral-associated trichodysplasia in a patient with lymphoma: a case report and review. *J Cutan Pathol.* 2007;34:721–725.
44. Lee JS, Frederiksen P, Kossard S. Progressive trichodysplasia spinulosa in a patient with chronic lymphocytic leukaemia in remission. *Australas J Dermatol.* 2008;49:57–60.
45. Holzer AM, Hughey LC. Trichodysplasia of immunosuppression treated with oral valganciclovir. *J Am Acad Dermatol.* 2009;60:169–172.
46. van der Meijden E, Janssens RW, Lauber C, et al. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromized patient. *Plos Pathog.* 2010;6:e1001024.
47. Schwieger-Briel A, Balma-Mena A, Ngan B, et al. Trichodysplasia spinulosa—a rare complication in immunosuppressed patients. *Pediatr Dermatol.* 2010;27:509–513.
48. Caccetta TP, Dessauvagie B, McCallum D, et al. Multiple minute digitate hyperkeratosis: a proposed algorithm for the digitate keratoses. *J Am Acad Dermatol.* 2012;67:e49–55.
49. Matthews MR, Wang RC, Reddick RL, et al. Viral-associated trichodysplasia spinulosa: a case with electron microscopic and molecular detection of the trichodysplasia spinulosa-associated human polyomavirus. *J Cutan Pathol.* 2011;38:420–431.
50. van der Meijden E, Kazem S, Burgers MM, et al. Seroprevalence of trichodysplasia spinulosa-associated polyomavirus. *Emerg Infect Dis.* 2011;17:1355–1363.
51. Chen T, Mattila PS, Jartti T, et al. Seroepidemiology of the newly found trichodysplasia spinulosa-associated polyomavirus. *J Infect Dis.* 2011; 204:1523–1526.
52. Tan BH, Busam KJ. Virus-associated trichodysplasia spinulosa. *Adv Anat Pathol.* 2011;18:450–453.

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53. Burns A, Arnason T, Fraser R, et al. Keratotic "spiny" papules in an immunosuppressed child. Trichodysplasia spinulosa (TS). *Arch Dermatol.* 2011;147:1215–1220.
54. Kazem S, van der Meijden E, Kooijman S, et al. Trichodysplasia spinulosa is characterized by active polyomavirus infection. *J Clin Virol.* 2012;53:225–230.
55. Fischer MK, Kao GF, Nguyen HP, et al. Specific detection of trichodysplasia spinulosa—associated polyomavirus DNA in skin and renal allograft tissues in a patient with trichodysplasia spinulosa. *Arch Dermatol.* 2012;148:726–733.
56. Brimhall CL, Malone JC. Viral-associated trichodysplasia spinulosa in a renal transplant patient. *Arch Dermatol.* 2012;148:863–864.
57. Elaba Z, Hughey L, Isayeva T, et al. Ultrastructural and molecular confirmation of the trichodysplasia spinulosa-associated polyomavirus in biopsies of patients with trichodysplasia spinulosa. *J Cutan Pathol.* 2012;39:1004–1009.
58. Kumar A, Kantele A, Järvinen T, et al. Trichodysplasia spinulosa-associated polyomavirus (TSV) and Merkel cell polyomavirus: correlation between humoral and cellular immunity stronger with TSV. *PLoS One*. 2012;7:e45773.
59. Rianthavorn P, Posuwan N, Payungporn S, et al. Polyomavirus reactivation in pediatric patients with systemic lupus erythematosus. *Tohoku J Exp Med.* 2012;228:197–204.
60. Wanat KA, Holler PD, Dentchev T, et al. Viral-associated trichodysplasia: characterization of a novel polyomavirus infection with therapeutic insights. *Arch Dermatol.* 2012;148:219–223.
61. Sadeghi M, Aronen M, Chen T, et al. Merkel cell polyomavirus and trichodysplasia spinulosa-associated polyomavirus DNAs and antibodies in blood among the elderly. *BMC Infect Dis.* 2012;12:383.
62. Feltkamp MC, Kazem S, van der Meijden E, et al. From Stockholm to Malawi: recent developments in studying human polyomaviruses. *J Gen Virol.* 2013;94:482–496.
63. Nicol JT, Robinot R, Carpentier A, et al. Age-specific seroprevalences of Merkel cell polyomavirus, human polyomaviruses 6, 7, and 9, and trichodysplasia spinulosa-associated polyomavirus. *Clin Vaccine Immunol.* 2013;20:363–368.
64. Lee YY, Tucker SC, Prow NA, et al. Trichodysplasia spinulosa: a benign adnexal proliferation with follicular differentiation associated with polyomavirus. *Australas J Dermatol.* 2014;55:e33–e36.
65. Kazem S, van der Meijden E, Feltkamp MC. The trichodysplasia spinulosa-associated polyomavirus: virological background and clinical implications. *APMIS.* 2013;121:770–782.
66. Moktefi A, Laude H, Brudy Gulphe L, et al. Trichodysplasia spinulosa associated with lupus. *Am J Dermatopathol.* 2014;36:e70–e74.
67. Berk DR, Lu D, Bayliss SJ. Trichodysplasia spinulosa in an adolescent with cystic fibrosis and lung transplantation. *Int J Dermatol.* 2013;52:1586–1588.
68. Diven DG. An overview of poxviruses. *J Am Acad Dermatol.* 2001;44:1–16.
69. Hawranek T, Tritscher M, Muss WH, et al. Feline orthopoxvirus infection transmitted from cat to human. *J Am Acad Dermatol.* 2003;49:513–518.
70. Smith KJ, Skelton HG III, James WD, et al. Parapoxvirus infections acquired after exposure to wildlife. *Arch Dermatol.* 1991;127:79–82.
71. Clark C, McIntyre PG, Evans A, et al. Human sealpox resulting from a seal bite: confirmation that sealpox virus is zoonotic. *Br J Dermatol.* 2005;152:791–793.
72. Dohil MA, Lin P, Lee J, et al. The epidemiology of molluscum contagiosum in children. *J Am Acad Dermatol.* 2006;54:47–54.
73. Inceoglu F. Orf (ecthyma contagiosum): an occasional diagnostic challenge. *Plast Reconstr Surg.* 2000;106:733–734.
74. Bodnar MG, Miller OF, Tyler WB. Facial orf. *J Am Acad Dermatol.* 1999;40:815–817.
75. Gurel MS, Ozardali I, Bitiren M, et al. Giant orf on the nose. *Eur J Dermatol.* 2002;12:183–185.
76. Gill MJ, Arlette J, Buchan KA, et al. Human orf, a diagnostic consideration? *Arch Dermatol.* 1990;126:356–358.
77. Groves RW, Wilson-Jones E, MacDonald DM. Human orf and milkers' nodule: a clinicopathologic study. *J Am Acad Dermatol.* 1991;25:706–711.
78. Li H, Ning Z, Hao W, et al. Identification and characterization of monoclonal antibodies against the ORFV059 protein encoded by Orf virus. *Virus Genes*. 2012;44:429–440.
79. de Vries RD, Mesman AW, Geijtenbeek TB, et al. The pathogenesis of measles. *Curr Opin Virol.* 2012;2:248–255.
80. Carrillo-Santisteve P, Lopalco PL. Measles still spreads in Europe: who is responsible for the failure to vaccinate? *Clin Microbiol Infect.* 2012;18(suppl 5):50–56.
81. Ackerman AB, Suringa DWR. Multinucleate epidermal cells in measles. *Arch Dermatol.* 1971;103:180–183.
82. Kimura A, Tosaka K, Nakao T. Measles rash, light and electron microscopic study of skin eruptions. *Arch Virol.* 1975;47:295–307.
83. Suringa DWR, Bank LJ, Ackerman AB. Role of measles virus in skin lesions and Koplik's spots. *N Engl J Med.* 1970;283:1139–1142.
84. Makino S, Yamaguchi F, Sata T, et al. The rash of measles is caused by a viral infection in the cells of skin: a case report. *J Dermatol.* 1994;21:741–745.
85. Yanagihara M, Fujii T, Mochizuki T, et al. Measles virus was present in the inner cell of the acrosyringium in the skin rash. *Pediatr Dermatol.* 1998;15:456–458.
86. Sheikine Y, Hawryluk EB, Burgin S, et al. Histopathology of measles exanthem: a case with characteristic features and eosinophils. *J Cutan Pathol.* 2012;39:667–670.
87. Kimura A, Tosaka K, Nakao T. An immunofluorescent and electron microscopic study of measles skin eruptions. *Tohoku J Exp Med.* 1975;117:245–256.
88. Odling-Stenkvist E, Bjorvatn B. Rapid detection of measles virus in skin rashes by immunofluorescence. *J Infect Dis.* 1976;134:463–469.
89. Moench TR, Griffin DE, Orbriecht CR, et al. Acute measles in patients with and without neurological involvement: distribution of measles virus antigen and RNA. *J Infect Dis.* 1988;158:433–442.
90. Yoshida M, Yamada Y, Kawahara K, et al. Development of follicular rash in measles. *Br J Dermatol.* 2005;153:1226–1228.
91. Tatsuo H, Ono N, Tanaka K, et al. SLAM (CDw150) is a cellular receptor for measles virus. *Nature*. 2000;406:893–897.
92. McQuaid S, Cosby SL. An immunohistochemical study of the distribution of the measles virus receptors, CD46 and SLAM, in normal human tissues and subacute sclerosing panencephalitis. *Lab Invest.* 2002;82:403–409.
93. Manchester M, Eto DS, Valsamakis A, et al. Clinical isolates of measles virus use CD46 as a cellular receptor. *J Virol.* 2000;74:3967–3974.
94. Cherry JD. Enteroviruses: polioviruses (poliomyelitis), coxsackieviruses, echoviruses and enteroviruses. In: Feigin RD, Cherry JD, eds. *Textbook of Pediatric Infectious Diseases*. Philadelphia, PA: WB Saunders; 1998:1787–1838.
95. Solomon T, Lewthwaite P, Perera D, et al. Virology, epidemiology, pathogenesis, and control of enterovirus 71. *Lancet Infect Dis.* 2010;10:778–790.
96. Park SH, Choi SS, Oh SA, et al. Detection and characterization of enterovirus associated with herpangina and hand, foot, and mouth disease in Seoul, Korea. *Clin Lab.* 2011;57:959–967.
97. Cherry JD, Bobinski JE, Horvath FL, et al. Acute hemangioma-like lesions associated with ECHO viral infections. *Pediatrics*. 1969;44:498–502.
98. Prose NS, Tope W, Miller SE, et al. Eruptive pseudoangiomatosis: a unique childhood exanthem? *J Am Acad Dermatol.* 1993;29:857–859.
99. Haneke E. Electron microscopic demonstration of virus particles in hand, foot and mouth disease. *Dermatologica*. 1985;171:321–326.
100. Zhang H, Li Y, Peng T, et al. Localization of enteroviral antigen in myocardium and other tissues from patients with heart muscle disease by an improved immunohistochemical technique. *J Histochem Cytochem.* 2000;48:579–584.
101. Samuelson A, Forsgren M, Sällberg M. Characterization of the recognition site and diagnostic potential of an enterovirus group-reactive monoclonal antibody. *Clin Diagn Lab Immunol.* 1995;2:385–386.
102. La Granade L, Manns A, Fletcher V, et al. Clinical, pathologic, and immunologic features of human T-lymphotropic virus type I-associated infective dermatitis in children. *Arch Dermatol.* 1998;134:439–444.
103. Ohshima K, Jaffe ES, Kikuchi M, et al. Adult T-cell leukaemia/lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue.* Lyon, France: IARC Press; 2008:281–284.
104. Amano M, Kurokawa M, Ogata K, et al. New entity, definition and diagnostic criteria of cutaneous adult T-cell leukemia/lymphoma:

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human T-lymphotropic virus type 1 proviral DNA load can distinguish between cutaneous and smoldering types. *J Dermatol.* 2008;35: 270–275.
105. Kato N, Sugawara H, Aoyagi S, et al. Lymphoma-type adult T-cell leukemia-lymphoma with a bulky cutaneous tumour showing multiple human T-lymphotropic virus-1 DNA integration. *Br J Dermatol.* 2001; 144:1244–1248.
106. Inoue M, Matsuoka M, Yamaguchi K, et al. Characterization of mRNA expression of IkappaB alpha and NF-kappaB subfamilies in primary adult T-cell leukemia cells. *Jpn J Cancer Res.* 1998;89:53–59.
107. Mulloy JC, Kislyakova T, Cereseto A, et al. Human T-cell lymphotropic/ leukemia virus type 1 Tax abrogates p53-induced cell cycle arrest and apoptosis through its CREB/ATF functional domain. *J Virol.* 1998;72: 8852–8860.
108. Pise-Masison CA, Radonovich M, Sakaguchi K, et al. Phosphorylation of p53: a novel pathway for p53 inactivation in human T-cell lymphotropic virus type 1-transformed cells. *J Virol.* 1998;72:6348–6355.
109. Suzuki T, Kitao S, Matsushime H, et al. HTLV-1 Tax protein interacts with cyclin-dependent kinase inhibitor p16INK4A and counteracts its inhibitory activity towards CDK4. *EMBO J.* 1996;15:1607–1614.
110. Tanimura A, Dan S, Yoshida M. Cloning of novel isoforms of the human Gli2 oncogene and their activities to enhance tax-dependent transcription of the human T-cell leukemia virus type 1 genome. *J Virol.* 1998;72:3958–3964.
111. Fan J, Ma G, Nosaka K, et al. APOBEC3G generates nonsense mutations in human T-cell leukemia virus type 1 proviral genomes in vivo. *J Virol.* 2010;84:7278–7287.
112. Okamoto T, Ohno Y, Tsugane S, et al. Multistep carcinogenesis model for adult T-cell leukemia. *Jpn J Cancer Res.* 1989;80:191–195.
113. Sasaki H, Nishikata I, Shiraga T, et al. Overexpression of a cell adhesion molecule, TSLC1, as a possible molecular marker for acute-type adult T-cell leukemia. *Blood*. 2005;105:1204–1213.
114. Ego T, Tanaka Y, Shimotohno K. Interaction of HTLV-1 Tax and methyl-CpG-binding domain 2 positively regulates the gene expression from the hypermethylated LTR. *Oncogene*. 2005;24:1914–1923.
115. Matsuoka M, Jeang KT. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat Rev Cancer.* 2007;7:270–280.
116. Hasui K, Wang J, Tanaka Y, et al. Development of ultra-super sensitive immunohistochemistry and its application to the etiological study of adult T-cell leukemia/lymphoma. *Acta Histochem Cytochem.* 2012;45: 83–106.
117. Peters CJ, Sanchez A, Rollin PE, et al. Filoviridae: Marburg and Ebola viruses. In: Fields BN, Knipe DM, Howley PM, et al, eds. *Fields Virology*. New York, NY: Lippincott-Raven; 1996:1161–1176.
118. Johnson KM, Lange JV, Webb PA, et al. Isolation and partial characterization of a new virus causing acute haemorrhagic fever in Zaire. *Lancet*. 1977;1:569–571.
119. Ksiazek TG, Rollin PE, Jahrling PB, et al. Enzyme immunosorbent assay for Ebola virus antigens in tissues of infected primates. *J Clin Microbiol.* 1992;30:947–950.
120. Zaki SR, Shieh W, Greer PW, et al. A novel immunohistochemical assay for the detection of Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. *J Infect Dis.* 1999; 179:S36–S47.

APPENDIX CME Examination

1. Which of the following entities are PVB19-related dermatologic diseases?
   a. Erythema infectiosum (fifth disease)
   b. Roseola infantum
   c. Purpuric “gloves-and-socks” syndrome (PPGSS)
   d. b and c
   e. a and c
2. To which of the following diseases has Merkel cell polyomavirus been related?
   a. Squamous cell carcinoma
   b. Merkel cell carcinoma
   c. Melanoma
   d. a and b
   e. b and c
3. Immunohistochemical studies have detected the measles virus in lesional skin within endothelial cells of:
   a. Dermal capillaries
   b. Epidermal multinucleate cells and lymphocytes
   c. Keratinocytes of the upper layers of the epidermis
   d. Hair follicles and sebaceous glands
   e. All of the above
4. Hand-foot-and-mouth disease (HFMD) is an exanthematous disease caused by:
   a. Herpes viruses
   b. Papillomaviruses
   c. Poxviruses
   d. Enteroviruses
   e. Parvoviruses
5. Which of the following monoclonal antibodies has proved higher sensitivity in detecting MCPyV large T antigen when used in skin biopsy specimens?
   a. CM2B4
   b. LNA-1
   c. CM5E1
   d. Ab3
   e. LMP1
6. Trichodysplasia spinulosa has been recently associated with a new virus included in the family of:
   a. Herpesviridae
   b. Papillomaviridae
   c. Polyomaviridae
   d. Parvoviridae
   e. Poxviridae

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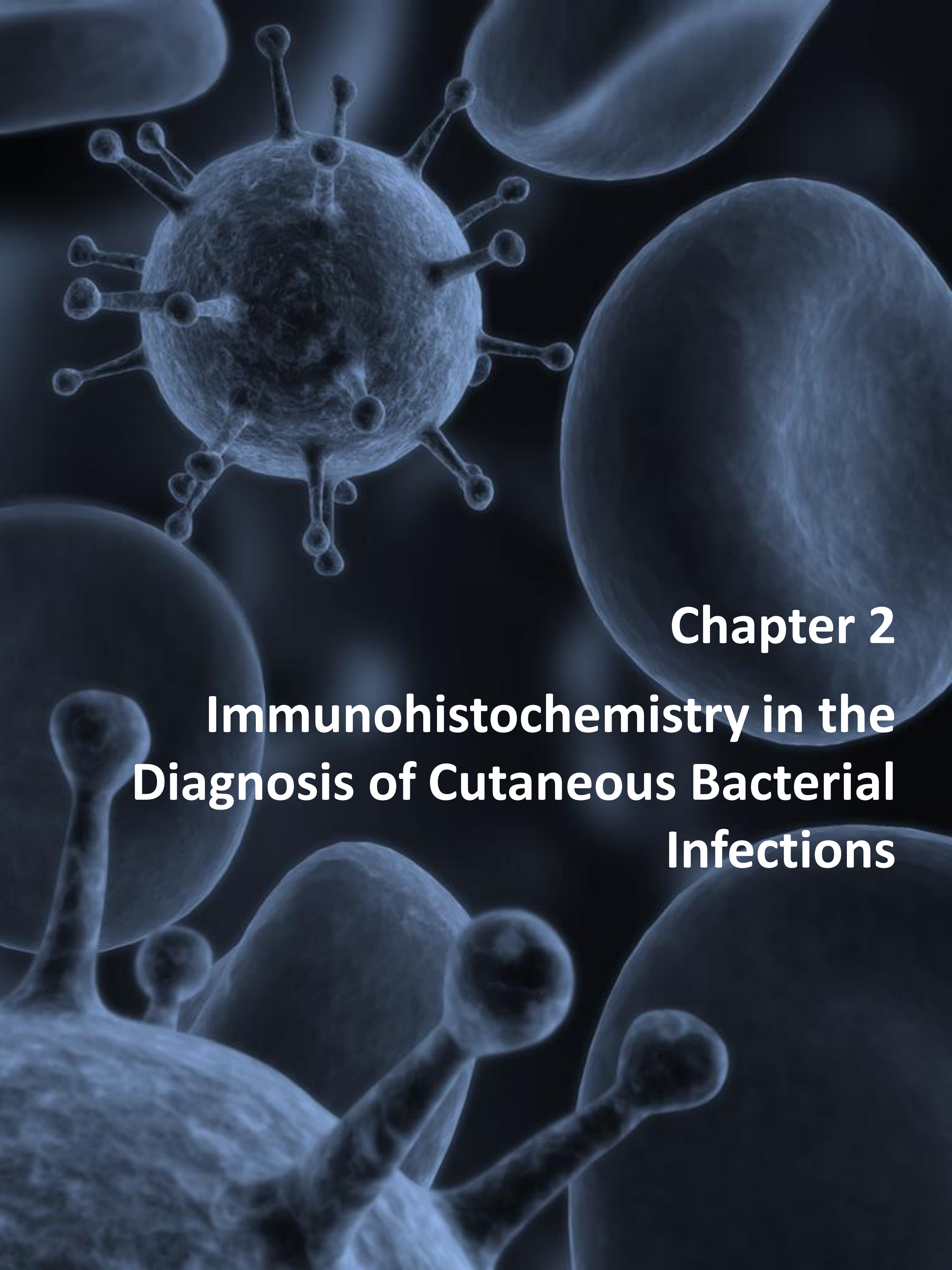

Chapter 2

Immunohistochemistry in the Diagnosis of Cutaneous Bacterial Infections

ARTICLE 3

Immunohistochemistry in the Diagnosis of Cutaneous Bacterial Infections

Immunohistochemistry in the Diagnosis of Cutaneous Bacterial Infections

Ana María Molina-Ruiz, MD, Lorenzo Cerroni, MD,† Heinz Kutzner, MD,‡ and Luis Requena, MD**

Abstract: The identification of pathogens is of vital importance for the adequate treatment of infections. During the past 2 decades, the approach to histopathologic diagnosis has been dramatically transformed by immunohistochemistry (IHC) specifically in the diagnosis and classification of tumors and more recently in the diagnosis of infectious diseases in tissue samples. The main goals of this article were to: (1) identify by IHC the cutaneous structures where bacterial pathogens are expressed in the course of infection, (2) identify the specific cells in which bacterial pathogens are expressed in positive cases, and (3) describe the pattern of distribution of the bacterial antigens within these cells (nuclear, cytoplasmatic, or membranous). This article is an up-to-date overview of the potential uses and limitations of IHC in the histopathologic diagnosis of cutaneous bacterial infections. In conclusion, IHC is especially useful in the identification of microorganisms that are present in low numbers, stain poorly, are fastidious to grow, culture is not possible, or exhibit an atypical morphology.

Key Words: immunohistochemistry, bacteria, skin, infection, pyogenic bacteria

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LEARNING OBJECTIVES

Upon completion of this learning activity, participants should be better able to:

1. Use immunohistochemistry (IHC) to identify bacterial pathogens that are relevant to dermatopathology.
2. Apply these techniques in the diagnosis of cutaneous bacterial infections and other related diseases.

INTRODUCTION

Traditionally, microbial identification in infectious diseases has been made primarily by using serologic assays and cultures. However, serologic results can be difficult to interpret in the setting of immunosuppression or when only a single sample is available for evaluation. In addition, fresh tissue is not always available for culture, and culture of certain pathogens can be difficult and may take weeks or months to yield results. Moreover, culture alone cannot distinguish colonization from tissue invasion. Some microorganisms have distinctive morphologic characteristics that allow their identification in formalin-fixed and paraffin-embedded (FFPE) tissues using routine and special stains. Nevertheless, in several instances, especially when the number of microorganisms is sparse, it is difficult or even impossible to identify an infectious agent specifically by conventional morphologic methods. Immunohistochemistry (IHC) has become an excellent tool in the diagnosis of cutaneous infections and plays an important role in recognizing infectious agents in tissue samples from patients, providing a rapid morphologic diagnosis and facilitating clinical decisions in patient treatment.

IHC uses antigen–antibody recognition in detecting specific antigens within tissues. The term covers a group of immunostaining techniques in which labeled antibodies are used to detect the presence of antigens in cells or tissues. The principle of IHC lies in the ability of antibodies to bind specifically to their respective antigens. The resulting reaction can only be visualized when the antibody is labeled with a substance that absorbs or emits light or produces color. Due to the technical advances, there has been a significant increase in the number of diagnostic IHC stains available for pathologists and dermatopathologists in recent years. The sensitivity and specificity of certain antibodies, their pattern of staining (nuclear, cytoplasmic, membranous, or different combinations of them), and the background artifact must be considered in their interpretation.

This article is an up-to-date overview of the potential uses and limitations of IHC in the histopathologic diagnosis of cutaneous bacterial infections. Emphasis is placed on new stains and novel uses of existing stains. For this purpose, we have studied several immunostains for specific bacteria that can be useful in dermatopathology focusing on 3 major objectives:

1. Identify by IHC the cutaneous structures where bacterial pathogens are expressed in the course of infection.
2. Identify the specific cells in which bacterial pathogens are expressed in positive cases.
3. Describe the pattern of distribution of the bacterial antigens within these cells (nuclear, cytoplasmatic, membranous, or combinations of them).

MATERIALS AND METHODS

The biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. For routine histology, 5-μm-thick sections were stained with hematoxylin and

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eosin. IHC stains were performed both at the Pathology Department of Fundación Jiménez Díaz University Hospital in Madrid and the Dermatopathology Laboratory at Friedrichshafen, Germany. Tissue sections were processed on a BioTek Solutions TechMate 500 (Dako) where 1-hour incubation with a primary antibody was performed. The IHC study of the bacterial pathogens was performed using the commercially available antibodies described in Table 1. In that table we also include the cross-reactivity with other microorganisms of some of these antibodies. Several antibodies for specific bacteria described in the literature are not commercially available at the time of elaboration of this manuscript; therefore, IHC staining for these pathogens could not be tested.

BACTERIAL PATHOGENS

Bacterial isolation is an important diagnostic tool in patients with skin and soft tissues infections, but culturing these tissues can be problematic. Sometimes, the isolation of certain bacteria can merely indicate colonization and the failure to isolate an organism may occur because of previous antibiotic treatment, suboptimal handling of specimens, or variable growth requirements.[1] IHC assays for bacteria are morphology-based techniques that use FFPE samples and can help define the pathogenic role of various microorganisms in the context of soft tissue infections.[2]

IHC on skin biopsy specimens has been shown through several studies to be useful in diagnosing cutaneous infections caused by pyogenic bacteria, *Clostridium* species, *Bacillus anthracis*, mycobacteria, spirochetes, rickettsiae, *Neisseria*, *Chlamydia*, *Bartonella*, *Helicobacter*, *Tropheryma*, etc. However, not all immunostains for these pathogens are commercially available nowadays because some of these stains have only been developed in private institutions for research purposes. In some cases, routine and/or histochemical staining is sufficient for diagnosis, and sometimes other laboratory methods may be more sensitive and cost-efficient. It is also important to remember that there may be common antigens among bacteria and pathogenic fungi and both monoclonal and polyclonal antibodies must be tested for possible cross-reactivity with other microorganisms (Table 1).

By using IHC assays for bacteria, we are able to identify intact bacteria and granular antigen staining. In some of these infections, particularly if antibiotic therapy has been given, bacterial antigens can be more abundant than intact bacteria, and in some cases, antigens can be the only evidence of the causative infectious agent. Interpretation of IHC assays may be difficult in cases where granular antigens are observed without intact bacteria, but in the appropriate clinicopathologic and epidemiological setting, granular antigen staining may be of important diagnostic value.[3]

PYOGENIC BACTERIA

Pyogenic infections, usually caused by *Staphylococcus aureus* and strains of *Streptococcus*, are numerically the most important bacterial infections of the skin.[4] Two distinct groups of pyogenic infections (superficial and deep) can be distinguished on the basis of the anatomic level of involvement of the skin. The pyogenic infections, with the exception of the staphylococcal "scalded skin" syndrome, which results from the effects of a bacterial exotoxin, are characterized histologically by a heavy infiltrate of neutrophils. These microorganisms may also infect hair follicles, resulting in folliculitis. Furuncles are deep-seated acute infections based on the folliculosebaceous

TABLE 1. Available Antibodies for Specific Bacteria Useful in Dermatopathology

Antibody Against	Type/Clone
Staphylococcus aureus	Anti-*S. aureus*, KPL BacTrace, polyclonal, goat; Medac
Streptococcus pyogenes (group A)	Anti-*Streptococcus* A, polyclonal, rabbit; Biorbyt
Clostridium species	Noncommercially available
Bacillus anthracis	Anti-anthrax PA/anthrax LF, polyclonal, rabbit; Xceltis
Mycobacterium bovis BCG (cross-reacts with other *Mycobacterium* species, including *M. tuberculosis*, *M. leprae*, *M. avium*, *M. phlei*, and *M. parafortuitum*)	Anti-*M. bovis* BCG, polyclonal, rabbit; DAKO
Mycobacterium tuberculosis (cross-reacts with other *Mycobacterium* species, including *M. leprae*, *M. avium*, *M. phlei*, and *M. parafortuitum*)	Anti-*M. tuberculosis*, polyclonal, rabbit; Zytomed Systems
Treponema pallidum (cross-reacts with *Borrelia burgdorferi*)	Anti-*T. pallidum*, polyclonal, rabbit; Zytomed Systems
Borrelia burgdorferi (Lyme disease) (cross-reacts with *T. pallidum*, *Borrelia hermsii*, *Borrelia parkeri*)	Anti-*Borrelia*, polyclonal, rabbit; Biogenesis
Borrelia species (specific for *B. burgdorferi*, *B. hermsii*, *Borrelia anserina*, and *Borrelia coriaceae*)	Anti-*Borrelia* species, KPL BacTrace, polyclonal, goat; Medac
Listeria species (cross-reacts with *Streptococcus* and *Staphylococcus*)	Anti-*Listeria* species, KPL BacTrace, polyclonal, goat; Medac
Chlamydia trachomatis (cross-reacts with *Chlamydia pneumoniae*, *Chlamydia psittaci*)	Anti-*C. trachomatis*, monoclonal (BDI815), mouse; Acris Antibodies
Bartonella henselae	Anti-*B. henselae*, monoclonal (H2A10), mouse; Zytomed Systems
Bartonella quintana	Anti-*B. quintana*; Xceltis
Rickettsia rickettsii; *Rickettsia akari*	Noncommercially available
Tropheryma whipplei	Noncommercially available
Neisseria meningitidis	Anti-*N. meningitidis*, polyclonal, rabbit; Thermo Fisher Scientific
Neisseria gonorrhoeae	Anti-*N. gonorrhoeae*, polyclonal, rabbit; Xceltis
Helicobacter pylori	Anti *H. pylori*, KPL BacTrace, polyclonal, goat; Medac
Francisella tularensis	Noncommercially available

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unit and adjacent dermis. IHC assays are useful for identification of pyogenic bacteria on FFPE samples of inflammatory soft tissue lesions and complement culture results by morphologically detecting the organism in areas with inflammation, which, in conjunction with the clinical features, will help defining if the microorganism is a pathogen or a commensal.

Staphylococcus aureus

Guarner et al[2] studied the application of IHC assays for *S. aureus* in FFPE tissue samples from patients with severe soft tissue infections. For this purpose, they used both a monoclonal anti-*S. aureus* IgG3 antibody (QED Bioscience, Inc), that reacted against the peptidoglycan of *S. aureus* (Fig. 1) and a polyclonal anti-*S. aureus* antibody (Biodesign) that reacted with *S. aureus* but did not cross-react against group A *Streptococcus* (GAS), group B *Streptococcus*, *Streptococcus pneumoniae*, *B. anthracis*, *Clostridium* species, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, or *Neisseria meningitidis*. By using IHC, they demonstrated bacteria and bacterial antigens in phagocytic cells at the portal of entry, including the affected skin, subcutaneous tissue, fascia, or muscle, indicating that the host had mounted an inflammatory reaction against the infectious agent and thus suggesting that the microorganism is a pathogen rather than just a commensal. They also studied the concordance of culture and IHC assay results in samples from 20 patients and found that *S. aureus* antigens were identified by IHC in wound tissues from 3 patients while only 1 of these 3 patients had positive cultures for *S. aureus*.

Group A *Streptococcus*

Streptococcus pyogenes (GAS) is the most frequent bacterial cause of tonsillitis, pharyngitis, and skin and soft tissue suppurative infections. Diagnosis of GAS infections is made when *S. pyogenes* is isolated from pus or body fluids. *Streptococcus pyogenes* might not be isolated if antibiotics were administered before culture samples were obtained or if symptoms are secondary to toxin production. Furthermore, samples often are not obtained for patients who die before seeking medical care. If autopsy or other specimens are available, diagnostic techniques that use FFPE samples and do not require viable bacteria, such as IHC or polymerase chain reaction (PCR), can be of great value.[5]

Guarner et al[5] were the first authors to report IHC assays for GAS using a polyclonal rabbit antibody against *S. pyogenes* (Biologics Branch, Centers for Disease Control) on FFPE tissue specimens from 122 patients with clinically or pathologically suspected GAS infection. Twenty-six cases of 122 patients showed IHC positivity for GAS, 5 of them having a mucocutaneous primary focus. Affected skin or mucosa from GAS infection showed various amounts of neutrophilic inflammation, marked edema, and necrosis of the dermis or submucosa, and adipose tissue septa. In these cases, GAS antigens were demonstrated in the surface and deep tissues. IHC showed the advantage of determining the distribution of cocci and GAS antigens, preserving morphologic features, and enabling a better understanding of how *S. pyogenes* caused the disease. Also, detection of GAS in the context of the morphologic features of tissue was helpful for determining the primary focus of infection and defining systemic spread of the disease, by localizing the bacteria in blood vessels of a variety of tissues. In addition, identification of streptococci inside inflammatory cells indicated that systemic spread occurred while the patient was alive rather than after death. Sometimes GAS may be found in superinfected preexisting lesions (Fig. 2).

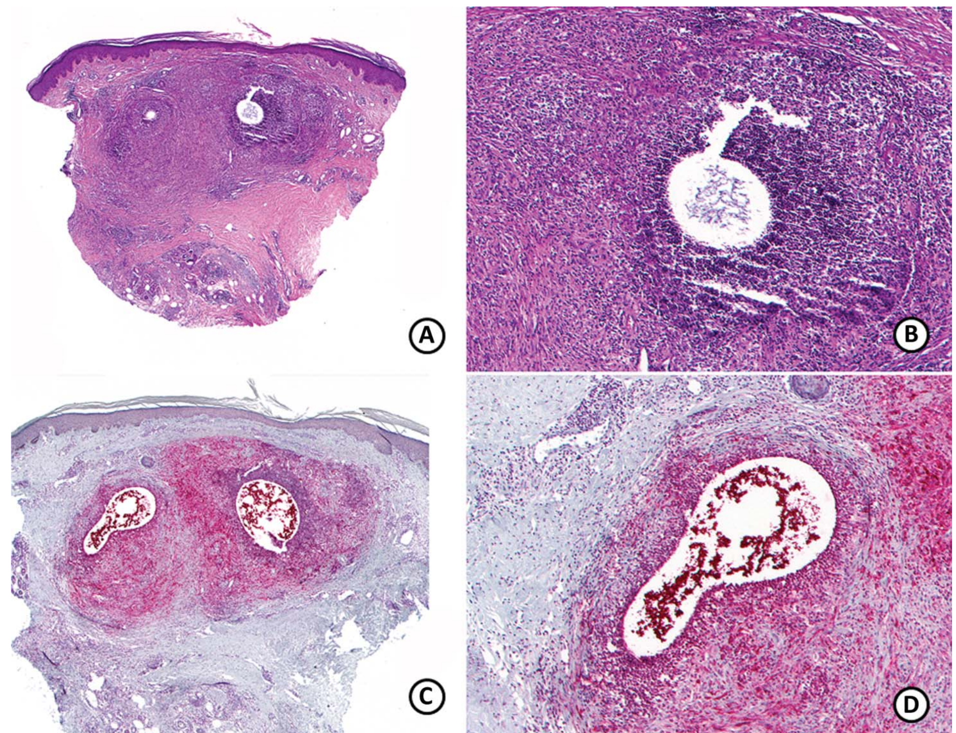

FIGURE 1. Histopathologic and immunohistochemical findings in a dermal abscess caused by *Staphylococcus aureus*. A, Scanning power showing nodular infiltrates in the dermis. B, Higher magnification showed suppurative granuloma. C, The same case immunohistochemically studied with monoclonal antibody against *S. aureus*. D, Higher magnification showed numerous positive microorganisms at the center of the suppurative granuloma.

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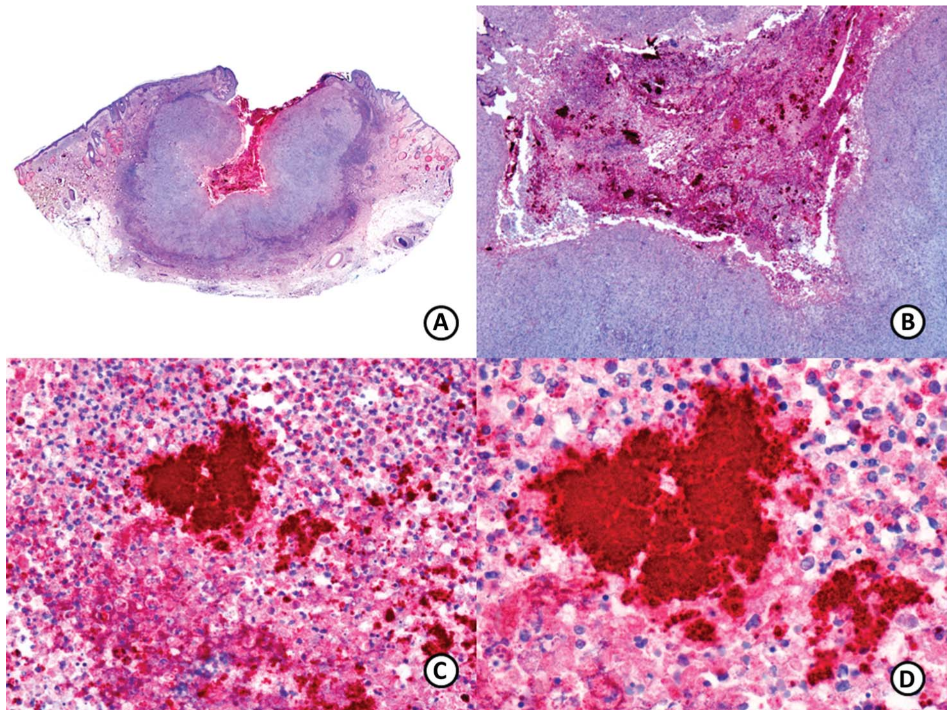

FIGURE 2. Immunohistochemical findings in a cutaneous SCC superinfected by *Streptococcus pyogenes*. A, Scanning power showed a well-demarcated crateriform neoplasm. B, Granular material and colonies of *S. pyogenes* were present in the central necrotic areas. C, Positive granular material and colonies of microorganisms are identified with the specific antibody against *S. pyogenes*. D, Higher magnification of a colony of *S. pyogenes*.

CLOSTRIDIUM SPECIES

Anaerobic spore-forming bacilli of the genus *Clostridium* are ubiquitous in the environment, existing in the form of exospores that may remain viable indefinitely. The genus *Clostridium* contains several species of known potential pathogenicity for man and animals, including the gas gangrene group *Clostridium perfringens*, *Clostridium septicum*, *Clostridium sordellii*, *Clostridium novyi* and *Clostridium histolyticum*. The contamination of wounds or illicit substances used by injecting drug users with clostridial species may result in gas gangrene (clostridial myonecrosis), leading to severe illness or death.[1]

IHC can be of great diagnostic value, particularly for bacteria such as *Clostridium* species, which are difficult to isolate because of their anaerobic fastidious growth requirements. Guarner et al[2] studied the application of IHC assays for *Clostridium* species on FFPE tissue samples from patients with severe soft tissue infections. For this purpose, they used the anti-*Clostridium* species antibody (Biodesign) that reacted with FFPE samples of *C. difficile*, *C. novyi*, *C. perfringens*, and *C. sordellii* but did not show reactivity on FFPE samples infected by GAS, group B *Streptococcus*, *B. anthracis*, *Bacillus cereus*, *Enterococcus* species, *S. aureus*, *S. pneumoniae*, *Listeria monocytogenes*, *Escherichia coli*, *K. pneumoniae*, or *P. aeruginosa*. They found that necrosis was frequently observed in clostridial IHC-positive cases (83%), and the IHC staining was focal and predominantly seen in areas with necrosis, edema, and inflammation. Clostridial IHC staining was primarily found in the portal of entry, whereas systemic bacterial presence was only documented as granular antigen staining in a locally draining lymph node, further supporting the role of toxins as the cause of systemic symptoms that can be present in patients with clostridial infections. Also, clostridial bacilli and clostridial antigens were observed inside inflammatory cells. However, the broad reactivity of the antibody against multiple *Clostridium* species has to be interpreted in the context of clinical and histopathologic findings because nonhistotoxic clostridia are components of the normal flora present on the skin and the gastrointestinal tract of humans.

Finally IHC assays have also been used to detect toxins, and some authors have localized the *C. perfringens* β2 toxin in the gastrointestinal tract of horses dying from typhlocolitis.[6]

BACILLUS ANTHRACIS

Cutaneous anthrax accounts for 95% of all naturally occurring *B. anthracis* infections in the United States.[6] Patients often have a history of occupational contact with animals or animal products contaminated with *B. anthracis* spores. These pathogenic spores are introduced through a cutaneous cut or abrasion, with the most common areas of exposure being the head, neck, and extremities. Bacteremia and toxemia following cutaneous infection can occur with a fatality rate of 20%–25% among untreated cases.[7] The clinical diagnosis traditionally has been established by conventional microbiological methods, such as culture and Gram staining. However, these methods often yield negative results when patients have received antibiotics.

Shieh et al[8] described the histopathologic features and the IHC findings of bioterrorism-related cutaneous anthrax cases of 2001 and compared the results of IHC assays with other laboratory diagnostic methods, including culture, special stains, PCR, and serology. For this purpose, the authors developed a colorimetric immunoalkaline phosphatase IHC method by using 2 monoclonal antibodies: (1) a mouse monoclonal IgM antibody reactive with *B. anthracis* cell wall antigen at 1:200 dilution (USA Military Research Institute of Infectious Disease) and (2) a mouse monoclonal IgM antibody reactive with *B. anthracis* capsule

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antigen at 1:1000 dilution. IHC staining revealed *B. anthracis* antigens in 80% of the cases, and all of them were positive by using both anti–cell wall and anti-capsule antibody. Extracellular bacilliform and granular antigens were distributed in both epidermis and dermis. Intracellular immunostaining was also observed in histiocytic cells and usually more prominent by anti-capsule antibody. The authors conclude that because IHC testing of skin biopsies can detect bacterial antigens in tissues regardless of the treatment, it provides a more sensitive and specific way to establish the diagnosis of cutaneous anthrax compared with other tests, including serology, culture, special stains, and PCR assay.

MYCOBACTERIAL INFECTIONS

The cutaneous mycobacterioses include tuberculosis (TB) and leprosy, as well as a diverse group of infections caused by various environmental (atypical, nontuberculous) mycobacteria.

Mycobacterium bovis Antibody (B124)

IHC staining with antibodies against *M. bovis*, the anti-bacillus Calmette–Guérin (BCG) polyclonal antibody (B124) proved to be an extremely valuable screening tool for the detection of bacteria or fungi in skin biopsy specimens. Original studies demonstrated that it recognized all mycobacteria and practically all gram-positive and gram-negative bacteria, as well as the spores and hyphae of dermatophytes and other fungi, whereas it did not recognize leishmaniasis or viral infections. It could perhaps be said that immunostaining with anti-BCG antibody produces similar results to those achieved with the combined use of Gram and periodic acid–Schiff (PAS) staining.[9]

Several studies showed that polyclonal anti-BCG antibodies cross-react with a broad spectrum of microorganisms. In 1972, Minden et al[10,11] first demonstrated shared antigens between *M. bovis* (BCG) and other bacterial species. In 1979, Harboe et al,[12] demonstrated cross-reactions between *M. bovis* BCG and various other mycobacteria, *Nocardia asteroides*, *Corynebacterium pyogenes* and *L. monocytogenes*. Later, Morris et al[13] identified antigenic determinants on *M. bovis* using monoclonal antibodies and found that the epitopes of mycobacterial antigens were similar to epitopes from other bacterial species. In the latter investigations, anti-BCG antibodies were successfully used for IHC detection of the most common cutaneous microbial pathogens in FFPE human tissue samples.[14,15]

In 1998, we studied a total of 254 FFPE skin specimens known to contain different species of microorganisms using a polyclonal rabbit anti-*M. bovis* (BCG) antibody (Dako code #B124; lot 063; titer 1:6000)[15] and demonstrated that anti-BCG labeled a wide spectrum of fungal and bacterial organisms with a high sensitivity and minimal background staining (Fig. 3) but did not cross-react with viruses, spirochetes, and protozoa. Regular skin structures, cellular debris, and necrotic material were not immunostained by anti-BCG, in contrast to most histochemical methods. In cases of fungal or bacterial infection, the results of the anti-BCG immunostain were clearly superior to those achieved with the conventional histochemical stains. Because of its minimal background staining, the anti-BCG immunostain was especially helpful in those cases in which few organisms were obscured by a dense overlying inflammatory infiltrate or in cases with scant microorganisms like panniculitis. Also, by labeling bacteria in the cytoplasm of polymorphonuclear granulocytes, it was possible to differentiate bullous impetigo from superficial pemphigus. However, because anti-BCG also labels physiologic skin flora (ie, mostly bacteria and pityrosporum in the follicular

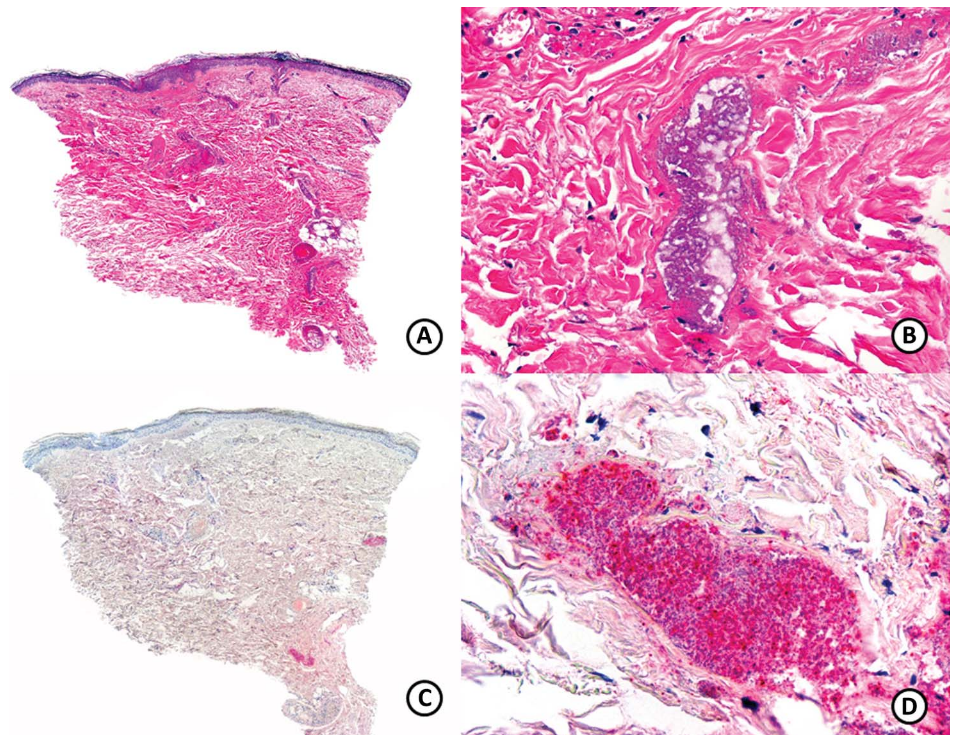

FIGURE 3. Histopathologic and immunohistochemical findings in a case of ecthyma gangrenosum caused by *Pseudomonas aeruginosa*. A, Scanning power showed dilated and congestive vessels at different levels of the dermis. B, Higher magnification demonstrated bluish granular material within the lumina of some vessels. These bluish granules corresponded to abundant bacteria of *P. aeruginosa*. C, The same case immunohistochemically stained with the anti-BCG polyclonal antibody (B124). D, Higher magnification demonstrated numerous bacteria of *P. aeruginosa* with strong immunostaining with anti-BCG antibody.

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infundibulum), this finding should not be overinterpreted as clinically significant.

Finally, this antibody has also proven to be useful in veterinary dermatopathology[16,17] and was found to be superior to histochemical staining methods as a general screening tool in cases where pathologic lesions suspicious for infections are evident and no microorganism can be cultured in vitro or only FFPE tissue samples are available for the laboratory examination.

However, due to overpurification by the manufacturer in recent years, B124 is no longer an "antibody for all seasons" that may be used for screening of bacterial and fungal infections, replacing the PAS and Gram staining, as originally was proposed, and currently the potential use of commercially available anti-BCG antibody should be restricted to the search of mycobacteria in FFPE samples.

Mycobacterium tuberculosis Complex

Mycobacterium tuberculosis complex members are causative agents of human and animal TB. Species in this complex include *M. tuberculosis*, the major cause of human TB, and many other mycobacteria, such as *M. bovis*, *M. bovis BCG*, *Mycobacterium africanum*, *Mycobacterium canetti*, *Mycobacterium caprae*, *Mycobacterium microti*, and *Mycobacterium pinnipedii*.

Ihama et al[18] recently investigated the utility of IHC staining with a species-specific IgG1 type mouse monoclonal antibody against the 38-kDa antigen of the *M. tuberculosis* complex (Vector Laboratories) to diagnose intestinal TB in archived FFPE intestinal tissue sections of suspected intestinal TB patients. Goel and Budhwar[19] also performed IHC localization of *M. tuberculosis* complex antigen and compared it with Ziehl–Neelsen staining in tissue granulomas of extrapulmonary TB. They found positivity in only 36.1% of tuberculous granulomas with Ziehl–Neelsen staining, whereas IHC staining was positive in 100% of tuberculous granulomata with zero false positivity and negativity. It is also our experience that this antibody is very helpful and more sensitive than acid-fast bacterial stains identifying *M. tuberculosis* in cutaneous TB lesions with few mycobacteria (Fig. 4), but its specificity is not absolute because it also stains other mycobacteria, both tuberculous and nontuberculous.

Mycobacterium leprae

The presence of mycobacterial antigens in leprosy skin lesions has been studied by immunohistological methods using monoclonal antibodies to *Mycobacterium leprae*–specific phenolic glycolipid I (PGL-I) and to cross-reactive mycobacterial antigens of 36 kDa, 65 kDa, and lipoarabinomannan (LAM).[20] The staining patterns with monoclonal antibodies to 36 and 65 kd were heterogeneous and were also seen in the lesions of other skin diseases. However, the in situ staining of mycobacterial cell wall–associated antigens (PGL-I and LAM) was seen only in leprosy. Both antigens were abundantly present intracellularly in CD68^{+} macrophages in the lesions of untreated multibacillary patients, whereas only PGL-I was occasionally seen in scattered macrophages in untreated paucibacillary lesions. These CD68^{+} macrophages containing mycobacterial antigens were frequently located adjacent to T cells and as scattered cells surrounding the granuloma. During treatment, clearance of PGL-I from granulomas in multibacillary lesions

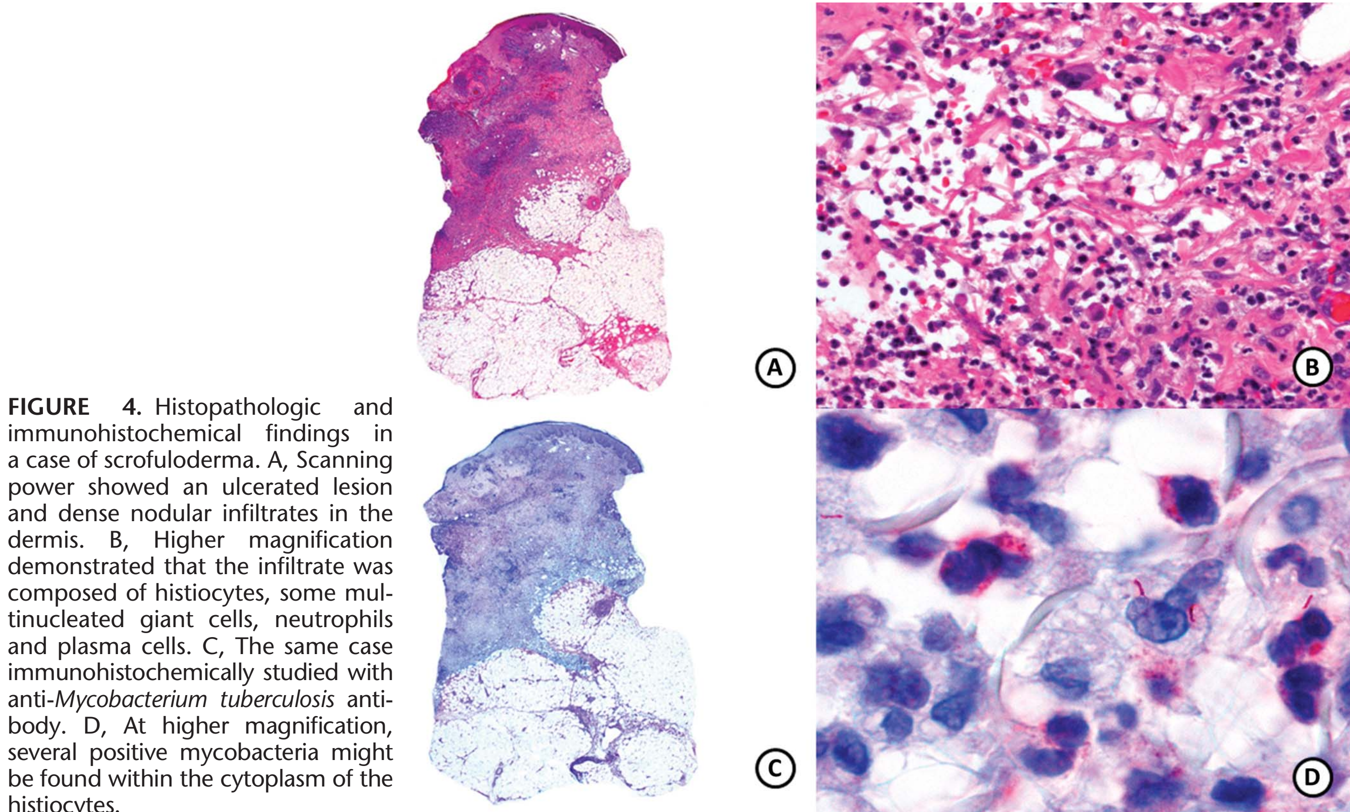

FIGURE 4. Histopathologic and immunohistochemical findings in a case of scrofuloderma. A, Scanning power showed an ulcerated lesion and dense nodular infiltrates in the dermis. B, Higher magnification demonstrated that the infiltrate was composed of histiocytes, some multinucleated giant cells, neutrophils and plasma cells. C, The same case immunohistochemically studied with anti-*Mycobacterium tuberculosis* antibody. D, At higher magnification, several positive mycobacteria might be found within the cytoplasm of the histiocytes.

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occurred before that of LAM, although the former persisted in scattered macrophages in some treated patients. The authors observed a differential expression pattern of PGL-I and LAM in the lesions of multibacillary patients with reactions during the course of the disease as compared with those without reactions and suggest that the in situ expression pattern of PGL-I and LAM in multibacillary patients may assist in early diagnosis of reactions versus relapse. As it has been stated above, *M. tuberculosis* antibody strongly stains *M. leprae* (Fig. 5).

Atypical (Nontuberculous) Mycobacteria

The atypical nontuberculous mycobacteria comprise a heterogeneous group of acid-fast bacteria, which differ from *M. tuberculosis* in their clinical manifestations, culture requirements, and sensitivity to anti-mycobacterial drugs.

Cutaneous infections by nontuberculous mycobacteria include specific entities, such as Buruli ulcer and swimming pool granuloma, and other miscellaneous group of infections caused by *Mycobacterium fortuitum*, *Mycobacterium chelonae-abscessus*, *Mycobacterium kansasii*, *Mycobacterium haemophilum*, *Mycobacterium scrofulaceum*, *Mycobacterium szulgai*, *Mycobacterium xenopi*, *Mycobacterium neoaurum*, *Mycobacterium gordonae*, and *Mycobacterium avium-intracellulare* complex.

Buruli ulcer is a chronic cutaneous and soft tissue infection by *Mycobacterium ulcerans*, which originates as a deep, painless, usually solitary ulcer with undermined edge, involving mostly distal areas of lower extremities of children and young adults in endemic areas of Central and West Africa, New Guinea, Australia, southeast Asia, and Mexico. Histopathology shows extensive coagulopathy necrosis involving the full thickness of the dermis and subcutis. Usually, there are numerous clumps of acid-fast bacilli in extracellular location. Although there is no commercially available specific antibody against *M. ulcerans*, Mwanatambwe et al[21] detected immunoreactivity for synthetic sugars of the terminal trisaccharide of *M. leprae* PGL-1M using mouse monoclonal antibodies in tissues infected with *M. ulcerans*. In our experience, *M. ulcerans* also express immunoreactivity with anti-BCG and anti-*M. tuberculosis* antibodies.

Swimming pool granuloma consists of a solitary verrucous nodule or plaque involving mostly dorsum of the hands or the forearms after superficial trauma in an aquatic environment caused by *Mycobacterium marinum*. Histopathologically, early lesions show suppurative granuloma, whereas more chronic lesions exhibit poorly formed noncaseating granulomas with variable epidermal changes. Acid-fast bacilli are found in a minority of cases, but their isolation is readily achieved by culture. Byrd et al[22] demonstrated immunohistochemical positivity for mycobacteria with anti-BCG antibody in cutaneous sporotrichoid lesions with positive culture for *M. marinum*.

Other nontuberculous mycobacteria produce a wide range of cutaneous lesions. The *M. fortuitum* and *M. chelonae/abscessus* group account for the majority of skin infections, including solitary verrucous nodules, ulcers, furuncles, abscesses, suppurative pannniculitis (Fig. 6) and spindle cell tumors. *Mycobacterium fortuitum* infections occur most commonly in healthy individuals, whereas infections by *M. chelonae/abscessus* group mostly develop in immunocompromised hosts. Several cases of disseminated infections caused by *M. avium-intracellulare* complex have been reported in AIDS patients. Cutaneous lesions in these patients are thought to be a manifestation of partial immune restoration after initiation of highly active antiretroviral therapy. Histopathology may show suppurative granuloma in rare cases, but more often cutaneous lesions exhibit a granulomatous infiltrate composed of voluminous histiocytes with granular cytoplasm, mimicking

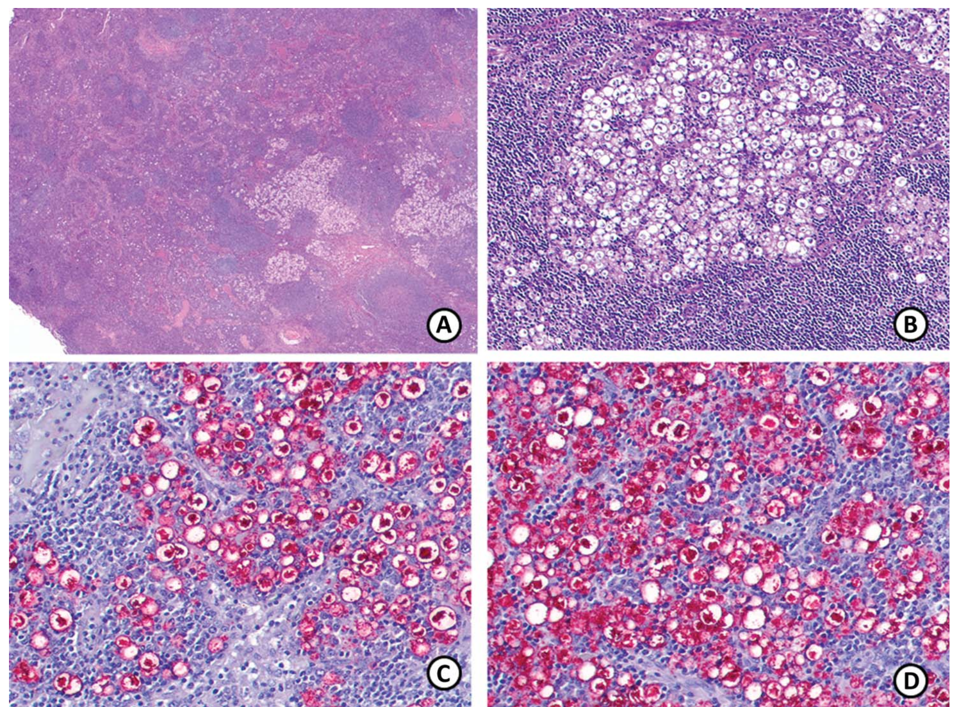
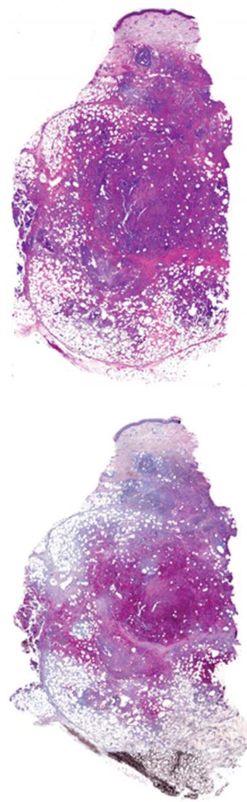


FIGURE 5. Histopathologic and immunohistochemical findings in a case of lepromatous leprosy. A, Dense diffuse infiltrate involving the dermis. B, Higher magnification demonstrated nodular aggregates of foamy histiocytes in the infiltrate. C, The same case stained with *Mycobacterium tuberculosis* antibody. D, *Mycobacterium leprae* showed strong cross reactivity with.



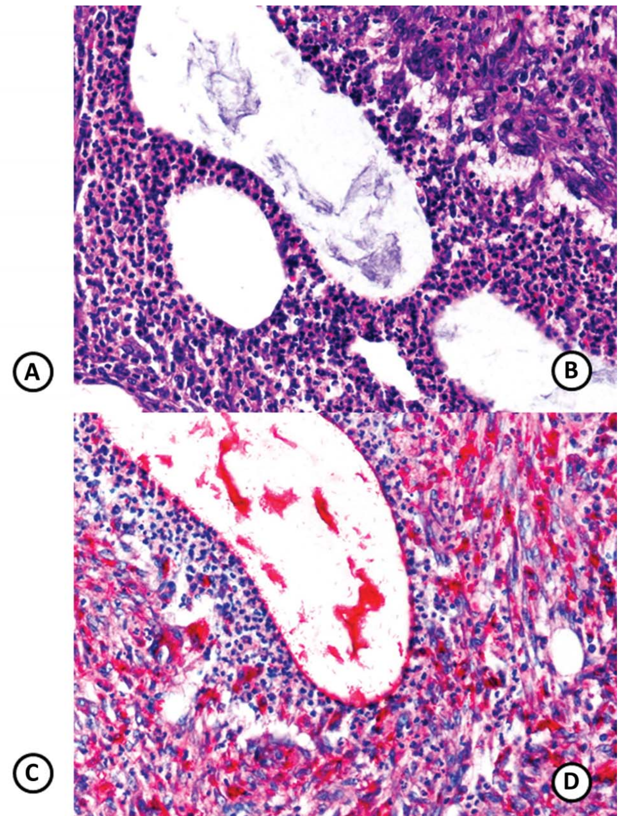


FIGURE 6. Histopathologic and immunohistochemical findings in a case of infective panniculitis caused by *Mycobacterium chelonae*. A, Scanning power showed a mostly lobular panniculitis. B, Higher magnification demonstrated suppurative granulomas with bluish material at the center, which corresponded to clusters of mycobacteria. C, The same case immunohistochemically studied with *Mycobacterium tuberculosis* antibody. D, At higher magnification numerous positive mycobacteria were found using *M. tuberculosis* antibody.

histoid leprosy or a spindle or granular cell tumor. In our experience, *M. avium-intracelluare* shows strong immunoreactivity with anti-BCG and anti-*M. tuberculosis* antibodies (Fig. 7).

SPIROCHETAL INFECTIONS

The order Spirochaetales has 2 genera of medical importance, *Treponema* and *Borrelia*.

Treponema pallidum

The treponematoses (syphilis, bejel, yaws, and pinta) are caused by infection with the spirochete *Treponema pallidum* and its various subspecies (*Treponema endemicum*, *Treponema pertenue*, *Treponema carateum*). The treponemes responsible for these different diseases are currently indistinguishable on morphologic and routine serological grounds and cannot be cultivated or maintained on artificial media.[23]

Treponema pallidum in FFPE tissue sections has classically been identified using silver impregnation staining techniques, such as the Levaditi or Warthin–Starry (WS) stains,[24] but immunoperoxidase techniques using polyclonal antibodies against *T. pallidum* improve spirochete visualization in FFPE preparations and have been proposed as a sensitive and useful method to avoid background staining and facilitate the identification and visualization of spirochetes in FFPE tissues.[25,26] Phelps et al[26] reported that immunoperoxidase for treponemes was more sensitive than conventional silver stain or serology.

In 2009, we participated in a study evaluating *T. pallidum* distribution patterns in mucocutaneous lesions of primary and secondary syphilis by IHC.[27] For this purpose, 34 biopsy specimens of patients with primary and secondary syphilis were stained with a specific polyclonal antibody against *T. pallidum* (Biocare Medical). IHC detected spirochetes in 80% of the biopsies and no background staining was observed. This study identified different patterns of *T. pallidum* distribution: (1) In primary syphilis, spirochetes were mainly distributed in the dermis or lamina propria and were arranged in a perivascular pattern surrounding and highlighting the vascular walls, adopting a peculiar pattern of distribution that was defined as a "vasculotropic pattern." Also, abundant *T. pallidum* with an intercellular distribution were noted within the lower layers of the epithelium adjacent to the ulceration that was defined as a "mixed vasculotropic-epitheliotropic pattern." (2) In secondary syphilis, spirochetes were mainly located within the lower layers of the epidermis, with an intercellular distribution that delineated the keratinocyte walls and was defined as an "epitheliotropic pattern" (Fig. 8). Scattered treponemes were also noted in more superficial epidermal layers and the papillary dermis. In 5 biopsy specimens, abundant *T. pallidum* spirochetes were distributed within the follicular infundibular epithelium and upper sweat gland duct epithelial cells.

In all biopsy specimens corresponding to primary syphilis, a consistently high density of treponemes was demonstrated, whereas a variable density of treponemes was found in those corresponding to secondary syphilis. In secondary syphilis, no relationship could be demonstrated between spirochete density and the evolution of the disease or the serological titers.

Finally, immunostaining with this anti-*T. pallidum* antibody should be interpreted with caution because it also stains

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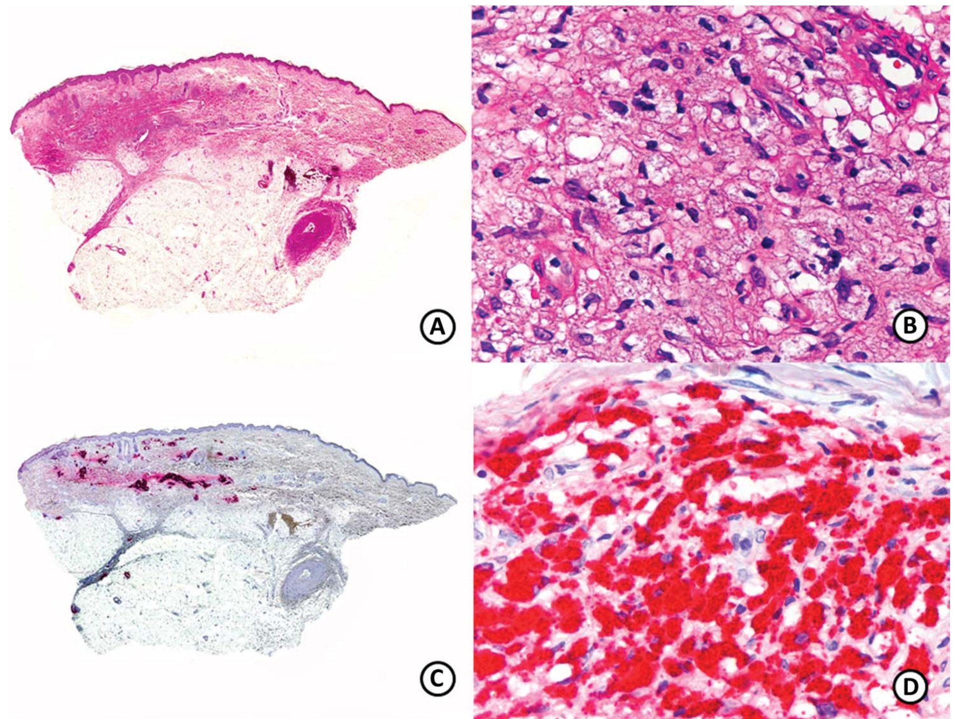

FIGURE 7. Histopathologic and immunohistochemical findings in a cutaneous lesion by *Mycocbacterium avium-intracellulare* in an AIDS patient. A, Scanning power showed discrete nodules of infiltrate in the dermis. B, At higher magnification, the nodules composed of aggregates of histiocytes with foamy or granular cytoplasm. C, The same case immunohistochemically stained with *M. tuberculosis* antibody. D, Higher magnification showing intense immunoreactivity for *M. tuberculosis* antibody within the cytoplasm of the histiocytes.

some acid–alcohol–resistant bacilli and *Helicobacter pylori*,[28] as well as some contaminant Treponema-positive bacteria that are present in the tap water used to wash the histologic slides. This latter event may be a pitfall especially in cutaneous biopsies with an infiltrate rich in plasma cells.[29]

Borrelia burgdorferi

The borrelioses are arthropod-borne infections, usually involving ticks of the genus *Ixodes*. Three genospecies of *Borrelia burgdorferi* have been identified as human pathogens (*Borrelia sensu stricto*, *Borrelia garinii*, and *Borrelia*

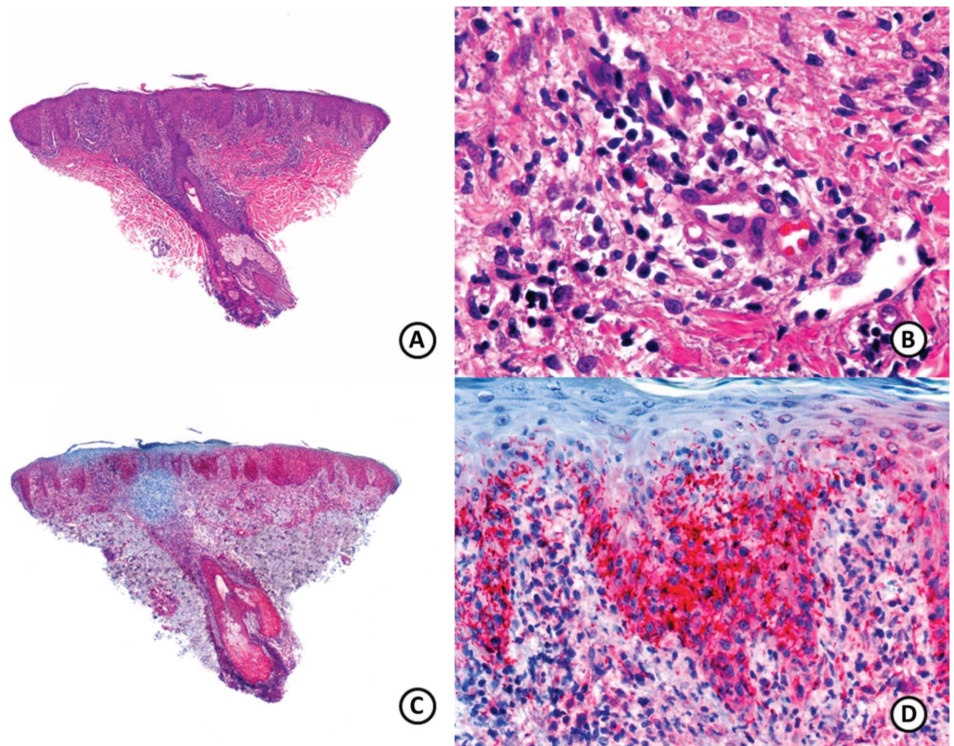

FIGURE 8. Histopathologic and immunohistochemical findings in a cutaneous lesion of secondary syphilis. A, Scanning power showed a lichenoid infiltrate. B, Higher magnification demonstrated that the infiltrate was mostly composed of lymphocytes and plasma cells. C, The same case immunohistochemically studied with a polyclonal antibody against *Treponema pallidum*. D, At higher magnification, numerous spirochetes were seen among the keratinocytes of the lower layers of the epidermis.

afzelii), which are involved in erythema migrans, acrodermatitis chronica atrophicans, *Borrelia*-associated lymphocytoma cutis, and *Borrelia*-associated B-cell lymphoma. *Borrelia* has also been suggested as a causative agent in a series of disparate disorders, including progressive facial hemiatrophy (Parry–Romberg disease),[30] eosinophilic fasciitis,[31] morphea, lichen sclerosus et atrophicus, atrophoderma of Pasini and Pierini,[32] and necrobiotic xanthogranuloma.[33,34]

Molecular detection of *Borrelia* in the skin has turned out to be difficult, and the results of direct detection of *Borrelia* by histochemical and IHC methods and culture are frequently negative. Studies by Aberer et al[35] and Neubert et al[36] confirmed that detection of *Borrelia* in tissue is usually a difficult, time-consuming, and often frustrating task. This is mainly due to the scant number of these spirochetes in many infections, their tiny structure (0.2 μm thickness), their intimate relationship to collagen bundles (which are 1–2 μm thick), and their variable appearance according to the relation to the section plane.

Focus floating microscopy has recently been proposed as the new "gold standard" for the diagnosis of cutaneous borreliosis.[37] This new IHC method involves staining sections with a *B. burgdorferi* antibody and then simultaneously scanning sections through 2 planes: horizontally in serpentines, as in routine cytology, and vertically by focusing through the thickness of the section (usually 3–4 μm), using standard histological equipment. The authors performed IHC staining in skin biopsies of cutaneous borreliosis with a polyclonal rabbit antibody (Acris BP1002, derived from immunization with whole-cell *B. burgdorferi* preparations strain B31, American Type Culture Collection No. 35210, reacting with 83- and 41-kDa flagellin, 32-kd OspB, and 31-kd OspA antigens and their fragments in Western blots, with cross-reaction to *T. pallidum*, *Borrelia hermsii*, and *Borrelia parkeri*). They found focus floating microscopy to be more sensitive than PCR (96.0% vs. 45.2%) and nearly equally specific. Spirochetes were observed surrounding the inflammatory process and were mostly single, occasionally paired, and delicate and, according to the section plane, very long and undulated, or comma-like to dot, and there was no milky cloud or veil. Within the inflammatory center, degenerative products of *Borrelia* such as swollen, granular, or clumped material could be found. Spirochetes or their degenerative products were frequently located along or between collagen bundles, partially or completely hidden if not visualized in the correct section plane. Occasionally, *Borrelia* were also seen within vessels, yet never in the epithelium of epidermis or adnexal structures.

CHLAMYDIAL INFECTIONS

Chlamydiae are obligate intracellular organisms that share many features with bacteria, including a discrete cell wall. There are 2 morphologically distinct species, *Chlamydia trachomatis* and *Chlamydia psittaci*.

Chlamydia trachomatis

Different serotypes of *C. trachomatis* are responsible for trachoma, urethritis, and lymphogranuloma venereum (LGV). Since 2003, there has been a considerable rise in the incidence of LGV in Western countries, almost exclusively in men who have sex with men. Proctitis has been by far the most common clinical manifestation of this infection in this scenario.[38,39]

Kumar et al[40] studied the sensitivity and reliability of immunodiagnostic assay for direct detection of *C. trachomatis* infections in endocervical specimens from female patients. For this purpose they developed species-specific monoclonal antibodies that recognized the major outer membrane protein of all serovars of *C. trachomatis*. They compared the reactivity of the developed species-specific monoclonal antibody with the commercially available direct fluorescent antibody test and found that the developed antibody had a higher sensitivity (97.22%) compared with direct fluorescent antibody for detection of chlamydial infection in endocervical samples and can be used as a reliable method in laboratories for the diagnosis of chlamydial infections.

We have recently studied 2 cases of LGV in men who have sex with men who presented with bubonulus involving the foreskin. IHC study using a monoclonal antibody reactive against *C. trachomatis* D/K and L2 serovars (Acris Antibodies catalog number AM00660PU-N) demonstrated the presence of the microorganisms within the cytoplasm of some histiocytes of the granulomatous areas of lesional skin (Fig. 9). In both cases, *C. trachomatis* particles were also detected by PCR on tissue. Serovar L2b was identified by PCR–restriction fragment length polymorphism genotyping.

Chlamydia psittaci

Psittacosis, caused by *C. psittaci*, usually presents as a pneumonic illness with various constitutional symptoms. Some patients develop a morbilliform rash or lesions resembling the rose spots of typhoid fever. Erythema nodosum,[41] erythema multiforme, erythema marginatum,[42] and disseminated intravascular coagulation[43] with cutaneous manifestations have also been reported. The findings are not specific for psittacosis, either clinically or histopathologically.

In 2011, Aigelsreiter et al[44] studied the association of *C. psittaci*, *Chlamydia pneumoniae*, and *C. trachomatis* with extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT). A total of 47 MALT lymphomas from nongastrointestinal sites, including the skin, were studied by both PCR and IHC for *C. psittaci*. IHC analysis for *C. psittaci* was performed on FFPE sections using a Chlamydiaceae family–specific mouse monoclonal antibody (Ab) directed against chlamydial lipopolysaccharide (clone ACI-P; Progen). *Chlamydia psittaci* was detected at variable frequencies in MALT lymphomas of different sites: 100% of lung lymphomas, 30% of thyroid gland lymphomas, 13% of salivary gland lymphomas, 15% of ocular adnexal lymphomas, and 25% of skin lymphomas. Together, nongastrointestinal MALT lymphomas were more frequently infected by *C. psittaci* than their respective nonmalignant control specimens. Positive reaction was exclusively found in macrophages, which have been shown by multiple detection methods to harbor viable and infectious *C. psittaci*.[45]

BARTONELLA

The genus *Bartonella* includes 19 distinct species, of which at least 6 are responsible for human disease (*Bartonella henselae*, *Borrelia bacilliformis*, *Bartonella quintana*, *Borrelia elizabethae*, *Borrelia vinsonii*, *Borrelia koehlerae*). These

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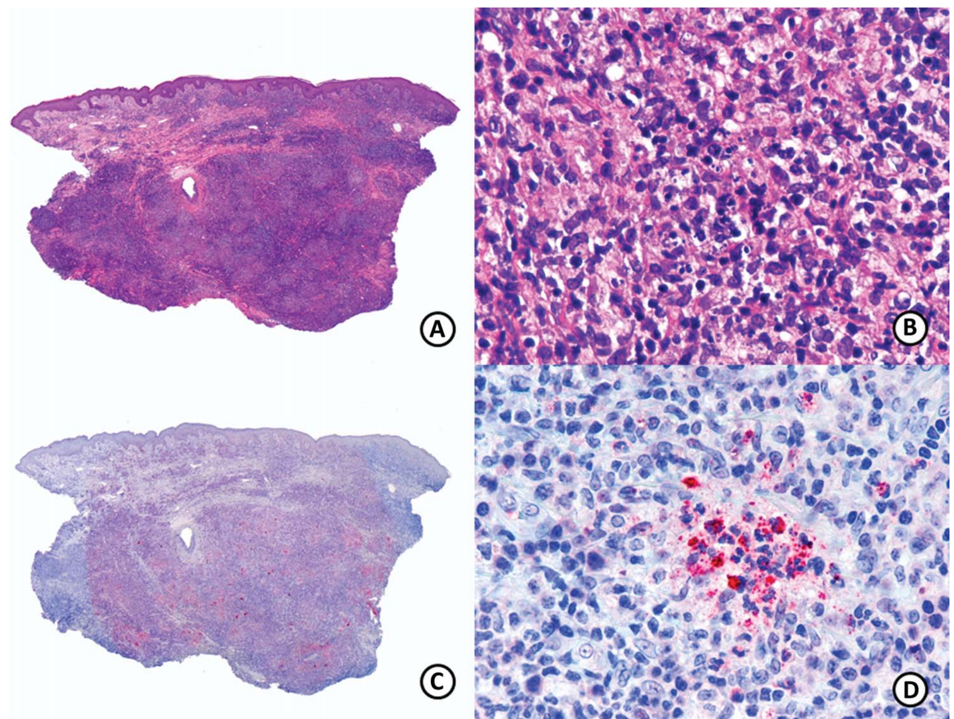

FIGURE 9. Histopathologic and immunohistochemical findings in a biopsy of LGV. A, Scanning power showed dense inflammatory infiltrate involving diffusely the dermis. B, At higher magnification, the infiltrate mostly composed of lymphocytes, plasma cells, histiocytes, and neutrophils. C, The same case immunohistochemically studied with a monoclonal antibody directed against chlamydial lipopolysaccharide. D, Higher magnification demonstrated *Chlamydia trachomatis* particles within the cytoplasm of some histiocytes of the infiltrate.

species are small, difficult to culture, intracellular gram-negative bacilli that are most easily visualized in tissue sections by using a WS silver impregnation stain or a Brown–Hopps tissue Gram stain.[46] *Bartonella henselae* has been established as the predominant pathogen in cat scratch disease, although *Borrelia clarridgeiae* has also been isolated from several cases.[47] Bacillary angiomatosis is caused by *B. henselae* and *B. quintana*,[48] and *B. quintana* is also the agent of trench fever.[49] Another strain of *Bartonella*, *B. bacilliformis* causes verruga peruana.[50]

Bartonella henselae

IHC demonstration of *B. henselae* in the diagnosis of cat scratch disease or bacillary angiomatosis had been reported by Min et al[51] and by Reed et al,[52] but the "home-made" polyclonal rabbit antiserum they used in their studies is not commercially available. In 2006, Cheuk et al[53] tested the utility of a commercially available monoclonal antibody against *B. henselae* in 6 cases of cat scratch disease, 3 cases of bacillary angiomatosis, and 24 cases of lymphadenopathy with reactive follicular and monocytoid B-cell hyperplasia. They performed immunostaining using the mouse monoclonal antibody clone H2A10 (Biocare Medical; 1:100 dilution) and found lack of staining in other tissue sections harboring bacillary and coccal bacteria, *H. pylori*, *M. tuberculosis*, *M. leprae*, *Cryptococcus*, *Aspergillus*, and intestinal spirochetosis confirming that there was no cross-reactivity against these microorganisms. In cat scratch disease, they found clumps of organisms within the necrotic foci, along proliferating vessels, and rarely within macrophages or monocytoid B cells. In bacillary angiomatosis, clusters of the microorganisms are seen with a granular appearance within the endothelial cells and extracellularly in the stroma of the capillary proliferation (Fig. 10). The bacteria are easy to identify because of the strong color contrast with the background. In comparison with WS stain, immunostaining revealed more bacteria, and interpretation was easier, especially where bacteria were present in low numbers.

Therefore, *B. henselae* antibody may be very helpful to identify *B. henselae* in lesions of bacillary angiomatosis and can aid in the distinction of cat scratch disease from other causes of suppurative granulomatous lymphadenitis such as tularemia, mycobacterial infection, brucellosis, fungal infection, and LGV. Immunostaining can also aid in the diagnosis of early lesions or atypical manifestations of cat scratch disease in the heart, bone, lung, solitary breast tumor, liver, spleen, and central nervous system. One potential pitfall, however, is that negative result is possible in the exceptional cases of cat scratch disease caused by *B. quintana* or *B. clarridgeiae*.[47] However, currently there also are commercially available antibodies against *B. quintana* (Table 1) and therefore distinction between *B. henselae* and *B. quintana* may be immunohistochemically performed in lesional tissue.

RICKETTSIAL INFECTIONS

Rickettsia are small obligate intracellular bacteria that are transmitted in most instances by the bite of an arthropod. The various species of *Rickettsia* are endemic in different geographical locations, and this has influenced the naming of some of the infections.[54,55] Cutaneous lesions are present in most rickettsial infections, with the exception of Q fever. Table 2 lists the etiology of the various rickettsial infections of humans.

Rickettsia rickettsii

The dermatologic diagnosis of Rocky Mountain spotted fever (RMSF) is often presumptive; the clinical presentation

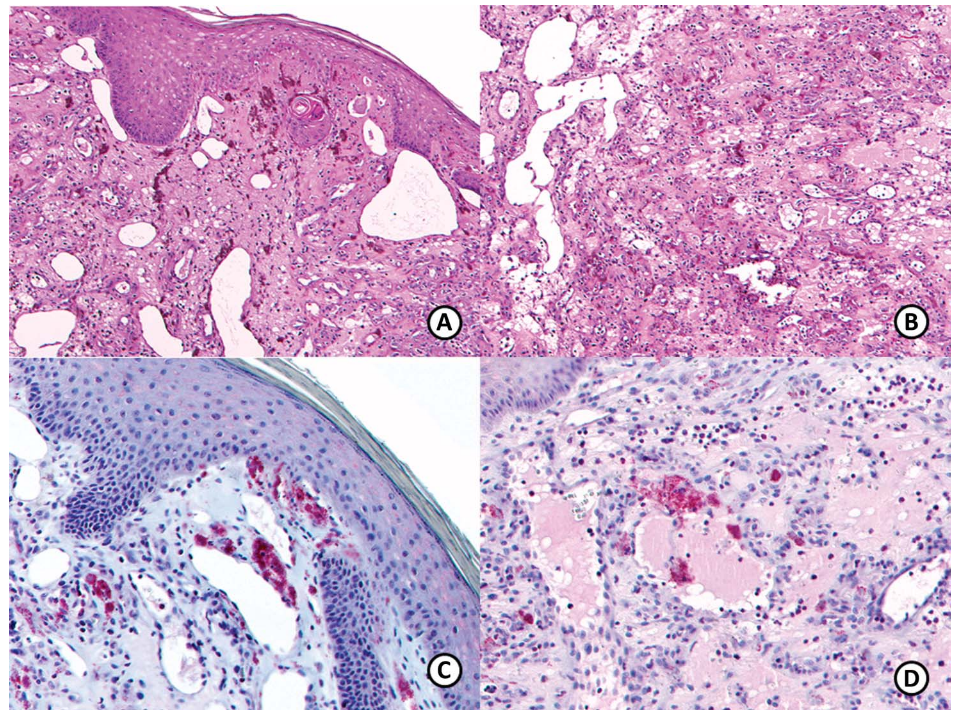

FIGURE 10. Histopathologic and immunohistochemical findings in a case of bacillary angiomatosis caused by *Bartonella henselae* in a patient with AIDS. A, Dilated vascular structures in the superficial dermis. B, In the deep dermis, the lesions showed the appearance of a vascular proliferation. C, The same case immunohistochemically studied with a monoclonal antibody against *B. henselae* demonstrated clusters of bacteria in the stroma close to the dilated vessels. D, Other area of positive clusters of *B. henselae* in the perivascular stroma.

includes skin rash and febrile illness with or without a clear history of tick bite. The cutaneous histopathology of RMSF is caused by endothelial damage by the rickettsial organisms that produce a septic vasculitis.

Demonstrating rickettsiae in patient tissues using conventional histochemical methods (Giemsa, Macchiavellos, and Pinkerton stains) is difficult to interpret and may produce misleading results.[56] Immunohistopathologic staining techniques for detecting *Rickettsia rickettsii* revolutionized the detection of these organisms in patient tissues and have been used since 1976. Studies have emphasized the clinical utility of immunostaining of skin biopsy specimens to guide patient care in suspected cases of RMSF or as a method to investigate the pathophysiology of RMSF by evaluating the anatomic distribution of rickettsial antigens in specific organs, including the skin.[57,58]

Several authors[59,60] have reported IHC detection of *R. rickettsii* with different, noncommercially available, polyclonal rabbit anti-*R. rickettsii* antibodies. Most reports describe fatal, serologically unconfirmed RMSF for whom a diagnosis of RMSF was established by IHC staining of tissues obtained at autopsy. Rickettsial antigens reactive with the anti-*R. rickettsii* antibody were visualized in multiple tissues from each patient including the skin. Rickettsiae were identified in or around small vessels in skin sections; however, the organisms were often sparse and distributed sporadically in the tissue. Rickettsial antigens and discrete coccobacilli were primarily located within the cytoplasm and occasionally in nuclei of infected endothelial cells; less frequently, they were located within macrophages and monocytes. Antimicrobial therapy may diminish the detection of rickettsiae by IHC staining, and scarcity of rickettsiae has been observed in skin biopsy specimens after 24–48 hours of tetracycline or chloramphenicol therapy.[61]

TABLE 2. Rickettsial Infections and Their Corresponding Etiology

Rickettsial Infection	Organism
Rocky Mountain spotted fever	*Rickettsia rickettsii*
Rickettsialpox	*Rickettsia akari*
Scrub typhus	*Orientia tsutsugamushi*
Q fever	*Coxiella burnetii*
Boutonneuse fever	*Rickettsia conorii*
African tick bite fever	*Rickettsia africae*
Siberian tick typhus	*Rickettsia sibirica*
Queensland tick typhus	*Rickettsia australis*
Epidemic typhus	*Rickettsia prowazakii*
Murine typhus	*Rickettsia mooseri*

Rickettsia akari

Rickettsialpox is a mild illness characterized by the appearance of a primary eschar at the site of a mite bite followed by fever, headache, and a papulovesicular rash. *Rickettsia akari*, the etiologic agent, belongs to the spotted fever group of rickettsial illnesses.

In the past, the laboratory diagnosis of rickettsialpox was based on testing of paired serum samples to detect a 4-fold increase in antibodies reactive with *R. akari*, a process requiring several weeks. More recently, direct immunofluorescence testing and IHC staining using an anti-*R. rickettsii* antibody have been used successfully to diagnose rickettsialpox using FFPE skin samples[60,62] due to cross-reactivity between *R. rickettsii* and *R. akari* because they both belong to the spotted fever group of rickettsia.[63,64]

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Orientia tsutsugamushi

Scrub typhus is an acute febrile disease characterized by high fever, headache, and rash. These symptoms are caused by the intracellular gram-negative bacteria *Orientia tsutsugamushi*. It is a major febrile disease in Korea, Japan, China, Thailand, etc.

Kim et al[65] studied the clinical usefulness of IHC staining on skin biopsy specimens for the diagnosis of scrub typhus compared with indirect immunofluorescent antibody assay (IFA), the "gold standard" for the definitive diagnosis of scrub typhus. For this purpose, they tested skin biopsy specimens from 63 patients with scrub typhus with ICR mouse polyclonal anti-*O. tsutsugamushi* antibody (dilution, 1:200) against the *O. tsutsugamushi* Boryong strain. They found that IHC staining of skin biopsy specimens, particularly that of eschars, is sensitive and specific and can be reliable for confirming the diagnosis of scrub typhus. They described that positive staining for *Orientia* antigens was most apparent within and associated with the vascular endothelium of capillaries, arterioles, venules, and veins. They observed intracytoplasmic positive staining of the lining epithelia of the sweat ducts and glands in the mid and deep dermis. *Orientia* antigens were primarily located within the cytoplasm of the infected endothelial cells and the macrophages around the blood vessels. Aggregates of fragmented, granular, or coalesced *Orientia* antigens were also demonstrated in some interstitial areas and more frequently in the areas of the more inflamed lesions. Finally, IHC positive staining was much more apparent for eschar lesions than for maculopapular lesions.

NEISSERIAL INFECTIONS

Primary infections of the skin by *N. meningitidis* and *Neisseria gonorrhoeae* are rare because these organisms are unable to penetrate intact epidermis. However, cutaneous lesions do occur quite commonly in meningococcal and gonococcal septicemia; they take the form of a septic vasculitis.

Neisseria meningitidis

Neisseria meningitidis is a strict human pathogen that is responsible for 3 major clinical syndromes, including chronic meningococcemia (the indolent form of the disease) and 2 life-threatening infections: cerebrospinal meningitis and purpura fulminans. The latter is a septic shock with disseminated intravascular coagulation and extensive dermal thrombosis leading to skin necrosis.

IHC demonstration of *N. meningitidis* in skin biopsy specimens of patients with purpura fulminans has been reported in the literature using several noncommercially available anti-*N. meningitidis* antibodies.[66] These studies have demonstrated that *N. meningitidis* targets human endothelial cells and that this interaction triggers the vascular damages that characterize purpura fulminans.[67] By IHC, they found microcolonies of *N. meningitidis* within leukocytes, small blood vessels, and the dermal interstitium.[68] The most frequent finding was extracellular bacteria adhering to the intraluminal surface of endothelial cells of capillaries and postcapillary venules of the skin and, in some circumstances, completely occluding the vascular lumen. Finally, they found no relationship between the severity of the clinical disease (as measured by Glasgow Meningococcal Prognostic Score) or the histological changes and the number of meningococci identified.

TROPHERYMA WHIPPLEI

Tropheryma whipplei is an obligate intracellular bacterium that causes Whipple disease (WD), which is a rare systemic infection clinically characterized by diarrhea, fever, weight loss, malabsorption, arthralgia, and, in some instances, cardiac and central nervous system manifestations. Cutaneous lesions in WD are infrequent, and the histological findings are usually nonspecific.[69] Specific cutaneous lesions have rarely been described and usually involve the subcutaneous fat.[70]

The diagnosis of WD is usually based on the demonstration of PAS-positive, diastase-resistant bacilli in tissue sections, but it may also be confirmed by PCR or by demonstration of characteristic bacillary bodies by electron microscopy.[71] IHC analysis using antibodies specifically directed against *T. whipplei* provides greater sensitivity and specificity than does PAS staining and is used to detect the organism in various tissues and bodily fluids and on blood monocytes.[72] Angelakis et al[73] tested 11 skin biopsies from patients with WD by PCR, culture, and PAS and IHC staining. They used a rabbit polyclonal antibody directed against *T. whipplei* that showed positive granular intracytoplasmic immunostaining within the macrophages in the skin. They also found that *T. whipplei* DNA is frequent in the skin biopsy of patients with classic WD without any apparent skin lesions and that the bacteria were alive in the skin, thus suggesting that the skin may be a reservoir for *T. whipplei*.

HELICOBACTER PYLORI

Helicobacter pylori is a gram-negative bacterium that is considered a causative agent of peptic ulcer disease, gastric lymphoma, and gastric carcinoma. An increasing number of dermatologic diseases have been related to *H. pylori* infection during the past decade, including rosacea, chronic idiopathic urticaria, psoriasis, Behçet disease, nodular prurigo, lichen planus, and palmoplantar pustulosis.[74] IHC demonstration of *H. pylori* has been reported in the literature using several commercially available anti-*H. pylori* antibodies in skin biopsy specimens of patients with prurigo pigmentosa,[75] squamous cell carcinoma,[76] oral lesions such as ulcerative/inflammatory lessions,[77] and primary lymphoma.[77] However, other studies have failed to detect immunohistochemically the presence of *H. pylori* in oral lichen planus.[78] Irani et al[77] tested 228 oral biopsies diagnosed as ulcerative/inflammatory lesions, squamous cell carcinoma, and primary lymphoma with a rabbit polyclonal anti-*H. pylori* antibody (Novocastra) and found that *H. pylori* positivity was seen in both the coccoid and the spiral forms and mostly detected in the epithelium (69.6%), followed by the lamina propria (33.4%), blood vessels (4.2%), salivary gland ducts, and the muscle layer of the tongue (0.3%). In addition, there was a statistically significant difference between the frequency of *H. pylori* positivity in normal tissues and the lesions examined. In the prurigo pigmentosa case reported, *H. pylori* positivity was mainly found within the dilated hair follicles.[73] Finally, it has also been described that *H. pylori* transforms into unculturable coccoid forms in unfavourable environments, resulting in

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the failure to detect this bacterium in oral samples by conventional stains like Giemsa[79] or culture techniques.[80] IHC, however, will still detect coccoid/posttreatment forms of *H. pylori*.

FRANCISELLA TULARENSIS

The gram-negative coccobacillus *F. tularensis* is the etiologic agent of tularemia, a disease that was classified in 2001 by the Centers for Disease Control into the most dangerous pathogen group, category A, along with smallpox and anthrax.[81] Skin lesions in tularemia occur only in the ulceroglandular form of the disease,[82] which is characterized by a papule at the site of the initial inoculation, followed by an ascending lymphangitis. The occurrence of erythema nodosum and erythema multiforme has been described.[83] The primary lesion is an ulcer, with necrosis involving the epidermis and upper dermis. The adjacent epidermis is usually acanthotic with some spongiosis. The causative organism cannot be seen in conventional histological preparations, but it can be demonstrated in FFPE skin biopsies by direct immunofluorescence and IHC. Asano et al[84] studied skin lesions of tularemia using an anti-*F. tularensis* antibody (Dr. Hotta, NIID, Japan) and found positive immunostaining for *F. tularensis* antigens mainly in abscesses and necrotic areas.

In summary, this article reviews the available immunohistochemical markers that are helpful to identify bacterial pathogens in cutaneous biopsies.

REFERENCES

1. Brazier J, Duerden B, Hall V, et al. Isolation and identification of *Clostridium* spp. from infections associated with the injection of drugs: experiences of a microbiological investigation team. *J Med Microbiol.* 2002;51:985–989.
2. Guarner J, Bartlett J, Reagan S, et al. Immunohistochemical evidence of *Clostridium* sp, *Staphylococcus aureus*, and group A *Streptococcus* in severe soft tissue infections related to injection drug use. *Hum Pathol.* 2006;37:1482–1488.
3. Guarner J, Shieh WJ, Morgan J, et al. Leptospirosis mimicking acute cholecystitis among athletes participating in a triathlon. *Hum Pathol.* 2001;32:750–752.
4. Weedon D. Bacterial and rickettsial infections. In: Weedon D, ed. *Weedon's Skin Pathology*. Elsevier; 2010:548–574.
5. Guarner J, Sumner J, Paddock CD, et al. Diagnosis of invasive group A streptococcal infections by using immunohistochemical and molecular assays. *Am J Clin Pathol.* 2006;12:148–155.
6. CDC. Update: investigation of anthrax associated with intentional exposure and interim public health guidelines, October 2001. *MMWR Morb Mortal Wkly Rep.* 2001;50:889–893.
7. Gold H. Anthrax: a report of 117 cases. *AMA Arch Intern Med.* 1955;96:387.
8. Shieh WJ, Guarner J, Paddock C, et al. Anthrax Bioterrorism Investigation Team. The critical role of pathology in the investigation of bioterrorism-related cutaneous anthrax. *Am J Pathol.* 2003;163:1901–1910.
9. Fuertes L, Santonja C, Kutzner H, et al. Immunohistochemistry in dermatopathology: a review of the most commonly used antibodies (part II). *Actas Dermosifiliogr.* 2013;104:181–203.
10. Minden P, McClatchy JK, Cooper R, et al. Shared antigens between *Mycobacterium bovis* (BCG) and other bacterial species. *Science*. 1972;176:57–58.
11. Minden P, McClatchy JK, Farr RS. Shared antigens between heterologous bacterial species. *Infect Immun.* 1972;6:574–582.
12. Harboe M, Mshana RN, Closs O, et al. Cross-reactions between mycobacteria. II. Crossed immunoelectrophoretic analysis of soluble antigens of BCG and comparison with other mycobacteria. *Scand J Immunol.* 1979;9:115–124.
13. Morris JA, Thorns CJ, Woolley J. The identification of antigenic determinants on *Mycobacterium bovis* using monoclonal antibodies. *J Gen Microbiol.* 1985;131:1825–1831.
14. Wiley EL, Beck B, Freeman RG. Reactivity of fungal organisms in tissue sections using anti-mycobacteria antibodies. *J Cutan Pathol.* 1991;18: 204–209.
15. Kutzner H, Argenyi ZB, Requena L, et al. A new application of BCG antibody for rapid screening of various tissue microorganisms. *J Am Acad Dermatol.* 1998;38:56–60.
16. Bonenberger TE, Ihrke PJ, Naydan DK, et al. Rapid identification of tissue micro-organisms in skin biopsy specimens from domestic animals using polyclonal BCG antibody. *Vet Dermatol.* 2001;12:41–47.
17. Szeredi L, Glávits R, Tenk M, et al. Application of anti-BCG antibody for rapid immunohistochemical detection of bacteria, fungi and protozoa in formalin-fixed paraffin-embedded tissue samples. *Acta Vet Hung.* 2008;56:89–99.
18. Ihama Y, Hokama A, Hibiya K, et al. Diagnosis of intestinal tuberculosis using a monoclonal antibody to *Mycobacterium tuberculosis*. *World J Gastroenterol.* 2012;18:6974–6980.
19. Goel MM, Budhwar P. Immunohistochemical localization of *Mycobacterium tuberculosis* complex antigen with antibody to 38 kDa antigen versus Ziehl Neelsen staining in tissue granulomas of extrapulmonary tuberculosis. *Indian J Tuberc.* 2007;54:24–29.
20. Verhagen C, Faber W, Klatser P, et al. Immunohistological analysis of in situ expression of mycobacterial antigens in skin lesions of leprosy patients across the histopathological spectrum. Association of *Mycobacterial lipoarabinomannan* (LAM) and *Mycobacterium leprae* phenolic glycolipid-I (PGL-I) with leprosy reactions. *Am J Pathol.* 1999;154:1793–1804.
21. Mwanatambwe M, Yajima M, Etuaful S, et al. Phenolic glycolipid-1 (PGL-1) in Buruli ulcer lesions. First demonstration by immunohistochemistry. *Int J Lepr Other Mycobact Dis.* 2002;70:201–205.
22. Byrd J, Mehregan DR, Mehregan DA. Utility of anti-bacillus Calmette-Guérin antibodies as a screen for organisms in sporotrichoid infections. *J Am Acad Dermatol.* 2001;44:261–264.
23. Liu H, Rodes B, George R, et al. Molecular characterization and analysis of a gene encoding acid repeat protein (Arp) of *Treponema pallidum*. *J Med Microbiol.* 2007;56:715–721.
24. Hoang M, High W, Molberg K. Secondary syphilis: a histologic and immunohistochemical evaluation. *J Cutan Pathol.* 2004;31:595–599.
25. Beckett J, Bigbec J. Immunoperoxidase localization of *Treponema pallidum*. *Arch Pathol Lab Med.* 1979;103:135–138.
26. Phelps RG, Knispel J, Tu ES, et al. Immunoperoxidase technique for detecting spirochetes in tissue sections: comparison with other methods. *Int J Dermatol.* 2000;39:609–613.
27. Martín-Ezquerra G, Fernandez-Casado A, Barco D, et al. *Treponema pallidum* distribution patterns in mucocutaneous lesions of primary and secondary syphilis: an immunohistochemical and ultrastructural study. *Hum Pathol.* 2009;40:624–630.
28. Fernandez-Flores A. Immunostaining for *Treponema pallidum*: caution in its evaluation. *Am J Dermatopathol.* 2010;32:523–526.
29. Fernandez-Flores A. Contaminant *Treponema*-positive bacteria in cutaneous biopsies with an infiltrate rich in plasma cells. *Am J Dermatopathol.* 2011;33:415–417.
30. Şahin MT, Bariş S, Karaman A. Parry-Romberg syndrome: a possible association with borreliosis. *J Eur Acad Dermatol Venereol.* 2004;18:204–207.
31. Granter SR, Barnhill RL, Duray PH. *Borrelia fasciitis*: diffuse fasciitis and peripheral eosinophilia associated with *Borrelia* infection. *Am J Dermatopathol.* 1996;18:465–473.
32. Hercogova J. Lyme borreliosis. *Int J Dermatol.* 2001;40:547–550.
33. Zelger B, Eisendle K, Mensing C, et al. Detection of spirochetal micro-organisms by focus-floating microscopy in necrobiotic xanthogranuloma. *J Am Acad Dermatol.* 2007;57:1026–1030.
34. White KP, Barry CI, Patterson JW. Focus-floating microscopy for detecting *Borrelia* species in tissue sections. Back to basics. *Arch Dermatol.* 2008;144:662–663.
35. Aberer E, Kersten A, Klade H, et al. Heterogeneity of *Borrelia burgdorferi* in the skin. *Am J Dermatopathol.* 1996;18:571–579.
36. Neubert U, Krampitz HE, Engl H. Microbiological findings in erythema (chronicum) migrans and related disorders. *Zentralbl Bakteriol Mikrobiol Hyg A.* 1986;263:237–252.
37. Eisendle K, Grabner T, Zelger B. Focus floating microscopy: "gold standard" for cutaneous borreliosis? *Am J Clin Pathol.* 2007;127:213–222.
38. Savage EJ, van de Laar MJ, Gallay A, et al. European surveillance of sexually transmitted infections (ESSTI) network. Lymphogranuloma venereum in Europe, 2003-2008. *Euro Surveill.* 2009;3:14.

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39. Richardson D, Goldmeier D. Lymphogranuloma venereum: an emerging cause of proctitis in men who have sex with men. *Int J STD AIDS.* 2007; 18:11–14.
40. Kumar A, Singh S, Salhan S, et al. Evaluation of a developed species-specific monoclonal antibody for detecting *Chlamydia trachomatis* infections in endocervical specimens from female patients. *Hybridoma (Larchmt).* 2007;26:333–337.
41. Sarner M, Wilson RJ. Erythema nodosum and psittacosis: report of five cases. *Br Med J.* 1965;2:1469–1470.
42. Semel JD. Cutaneous findings in a case of psittacosis. *Arch Dermatol.* 1984;120:1227–1229.
43. Green ST, Hamlet NW, Willocks L, et al. Psittacosis presenting with erythema marginatum-like lesions—a case report and a historical review. *Clin Exp Dermatol.* 1990;15:225–227.
44. Aigelsreiter A, Gerlza T, Deutsch AJ, et al. *Chlamydia psittaci* infection in nongastrointestinal extranodal MALT lymphomas and their precursor lesions. *Am J Clin Pathol.* 2011;135:70–75.
45. Ferreri AJ, Guidoboni M, Ponzoni M, et al. Evidence for an association between *Chlamydia psittaci* and ocular adnexal lymphomas. *J Natl Cancer Inst.* 2004;96:586–594.
46. Florin TA, Zaoutis TE, Zaoutis LB. Beyond cat scratch disease: widening spectrum of *Bartonella henselae* infection. *Pediatrics*. 2008;121:1413–1425.
47. Mehmi M, Lim SP, Tan CY. An unusual cutaneous presentation of cat-scratch disease. *Clin Exp Dermatol.* 2007;32:219–220.
48. Nosal JM. Bacillary angiomatosis, cat-scratch disease, and bartonellosis: what's the connection? *Int J Dermatol.* 1997;36:405–411.
49. Guibal F, de la Salmonière P, Rybojad M, et al. High seroprevalence to *Bartonella quintana* in homeless patients with cutaneous parasitic infestations in downtown Paris. *J Am Acad Dermatol.* 2001;44:219–223.
50. Arias-Stella J, Lieberman PH, Erlandson RA, et al. Histology, immunohistochemistry, and ultrastructure of the verruga in Carrion's disease. *Am J Surg Pathol.* 1986;10:595–610.
51. Min K, Reed J, Welch D, et al. Morphologically variable bacilli of cat scratch disease are identified by immunocytochemical labeling with antibodies to *Rochalimaea henselae*. *Am J Clin Pathol.* 1994;101:607–610.
52. Reed J, Brigati D, Flynn SD, et al. Immunocytochemical identification of *Rochalimaea henselae* in bacillary (epithelioid) angiomatosis, parenchymal bacillary peliosis, and persistent fever with bacteremia. *Am J Surg Pathol.* 1992;16:650–657.
53. Cheuk W, Chan AK, Wong MC, et al. Confirmation of diagnosis of cat scratch disease by immunohistochemistry. *Am J Surg Pathol.* 2006;30: 274–275.
54. Boyd AS, Neldner KH. Typhus disease group. *Int J Dermatol.* 1992;31: 823–832.
55. Kim J, Smith KJ, Naefie R, et al. Histopathologic features of and lymphoid populations in the skin of patients with the spotted fever group of rickettsiae: southern Africa. *Int J Dermatol.* 2004;43:188–194.
56. Walker DH. Diagnosis of rickettsial diseases. *Pathol Annu.* 1988;23:69–96.
57. Dumler JS, Gage WR, Pettis GL, et al. Rapid immunoperoxidase demonstration of *Rickettsia rickettsii* in fixed cutaneous specimens from patients with Rocky Mountain spotted fever. *Am J Clin Pathol.* 1990; 93:410–411.
58. White WL, Patrick JD, Miller LR. Evaluation of immunoperoxidase techniques to detect Rickettsia rickettsii in fixed tissue sections. *Am J Clin Pathol.* 1994;101:747–752.
59. Kao GF, Evancho CD, Ioffe O, et al. Cutaneous histopathology of Rocky Mountain spotted fever. *J Cutan Pathol.* 1997;24:604–610.
60. Paddock CD, Greer PW, Ferebee TL, et al. Hidden mortality attributable to Rocky Mountain spotted fever: immunohistochemical detection of fatal, serologically unconfirmed disease. *J Infect Dis.* 1999;179:1469–1476.
61. Walker DH, Cain BG, Olmstead PM. Laboratory diagnosis of Rocky Mountain spotted fever by immunofluorescent demonstration of Rickettsia rickettsii in cutaneous lesions. *Am J Clin Pathol.* 1978;69:619–623.
62. Sanders S, Di Costanzo D, Leach J, et al. Rickettsialpox in a patient with HIV infection. *J Am Acad Dermatol.* 2003;48:286–289.
63. Hébert GA, Tzianabos T, Gamble WC, et al. Development and characterization of high-titered, group-specific fluorescent-antibody reagents for direct identification of rickettsiae in clinical specimens. *J Clin Microbiol.* 1980;11:503–507.
64. Koss T, Carter EL, Grossman ME, et al. Increased detection of rickettsialpox in a New York City hospital following the anthrax outbreak of 2001: use of immunohistochemistry for the rapid confirmation of cases in an era of bioterrorism. *Arch Dermatol.* 2003;139:1545–1552.
65. Kim DM, Park CJ, Lim SC, et al. Diagnosis of scrub typhus by immunohistochemical staining of *Orientia tsutsugamushi* in cutaneous lesions. *Am J Clin Pathol.* 2008;130:543–551.
66. Gidney MA, Plested JS, Lacelle S, et al. Development, characterization, and functional activity of a panel of specific monoclonal antibodies to inner core lipopolysaccharide epitopes in *Neisseria meningitidis*. *Infect Immun.* 2004;72:559–569.
67. Join-Lambert O, Lecuyer H, Miller F, et al. Meningococcal interaction to microvasculature triggers the tissular lesions of purpura fulminans. *J Infect Dis.* 2013;208:1590–1597.
68. Harrison OB, Robertson BD, Faust SN, et al. Analysis of pathogen-host cell interactions in purpura fulminans: expression of capsule, type IV pili, and PorA by *Neisseria meningitidis* in vivo. *Infect Immun.* 2002;70: 5193–5201.
69. Schaller J, Carlson JA. Erythema nodosum-like lesions in treated Whipple's disease: signs of immune reconstitution inflammatory syndrome. *J Am Acad Dermatol.* 2009;60:277–288.
70. Canal L, Fuente Dde L, Rodriguez-Moreno J, et al. Specific cutaneous involvement in Whipple disease. *Am J Dermatopathol.* 2014;36:344–346.
71. Baisden BL, Lepidi H, Raoult D, et al. Diagnosis of Whipple disease by immunohistochemical analysis: a sensitive and specific method for the detection of *Tropheryma whipplei* (the Whipple bacillus) in paraffin-embedded tissue. *Am J Clin Pathol.* 2002;118:742–748.
72. Fenollar F, Puechal X, Raoult D. Whipple's disease. *N Engl J Med.* 2007; 356:55–66.
73. Angelakis E, Fenollar F, Lepidi H, et al. Tropheryma whipplei in the skin of patients with classic Whipple's disease. *J Infect.* 2010;61:266–269.
74. Tüzün Y, Keskin S, Kote E. The role of *Helicobacter pylori* infection in skin diseases: facts and controversies. *Clin Dermatol.* 2010;28:478–482.
75. Missall TA, Pruden S, Nelson C, et al. Identification of Helicobacter pylori in skin biopsy of prurigo pigmentosa. *Am J Dermatopathol.* 2012;34:446–448.
76. Grimm M, Munz A, Exarchou A, et al. Immunohistochemical detection of Helicobacter pylori without association of TLR5 expression in oral squamous cell carcinoma. *J Oral Pathol Med.* 2014;43:35–44.
77. Irani S, Monsef Esfahani A, Bidari Zerehpoush F. Detection of Helicobacter pylori in oral lesions. *J Dent Res Dent Clin Dent Prospects.* 2013; 7:230–237.
78. Hulimavu SR, Mohanty L, Tondikulam NV, et al. No evidence for Helicobacter pylori in oral lichen planus. *J Oral Pathol Med.* 2014. Epub ahead of print.
79. Wabinga HR. Comparison of immunohistochemical and modified Giemsa stains for demonstration of Helicobacter pylori infection in an African population. *Afr Health Sci.* 2002;2:52–55.
80. Shahamat M, Alavi M, Watts JE, et al. Development of two PCR-based techniques for detecting helical and coccoid forms of Helicobacter pylori. *J Clin Microbiol.* 2004;42:3613–3619.
81. Inglesby TV, O'Toole T, Henderson DA, et al. Anthrax as a biological weapon: updated recommendations for management. *JAMA*. 2002;287: 2236–2252.
82. Cerny Z. Skin manifestations of tularemia. *Int J Dermatol.* 1994;33:468–470.
83. Akdis AC, Kiliçturgay K, Helvaci S, et al. Immunological evaluation of erythema nodosum in tularaemia. *Br J Dermatol.* 1993;129:275–279.
84. Asano S, Mori K, Yamazaki K, et al. Temporal differences of onset between primary skin lesions and regional lymph node lesions for tularemia in Japan: a clinicopathologic and immunohistochemical study of 19 skin cases and 54 lymph node cases. *Virchows Arch.* 2012;460:651–658.

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CME EXAMINATION

1. The antibody against *Treponema pallidum* shows cross reactivity with:

a. *Staphylococcus aureus*
b. *Helicobacter pylori*
c. *Pseudomona aeruginosa*
d. *Bartonella henselae*
e. *Streptococcus pyogenes*

2. The strongest immunohistochemical staining of *Treponema pallidum* in secondary syphilis is seen:

a. Around the vascular walls of the papillary dermis
b. Around the vascular walls of the reticular dermis
c. Between the adipocytes
d. Around the eccrine glands
e. In the lower layers of the epidermis

3. Focus floating microscopy (FFM) has recently been proposed as the new "gold standard" for the diagnosis of:

a. Tuberculosis
b. Whipple disease
c. Cutaneous borreliosis
d. Cutaneous tularemia
e. Purpura fulminans

4. Which of the following bacteria are the etiologic agents of bacillary angiomatosis?

a. *B. henselae* and *B. quintana*
b. *B. bacilliformis* and *B. quintana*
c. *B. elizabethae* and *B. vinsonii*
d. *B. koehlerae* and *B. henselae*
e. *B. henselae* and *B. vinsonii*

5. Immunohistochemical demonstration of *Neisseria meningitidis* in skin biopsy specimens of patients with purpura fulminans has demonstrated that *N. meningitidis* mainly targets:

a. Neutrophils
b. Histiocytes
c. Endothelial cells
d. Lymphocytes
e. Adipocytes

6. Which of the following bacteria have been used in bioterrorism?

a. *Bacillus anthracis*
b. *Chlamydia trachomatis*
c. *Helicobacter pylori*
d. *Francisella tularensis*
e. a and d

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Conclusions

Conclusions

1. A total of 25 different specific commercially available antibodies were identified and tested in cutaneous biopsy specimens from patients with a wide range of cutaneous viral and bacterial infections.

2. IHC was especially useful in the identification of microorganisms that are (1) difficult to detect by routine or special stains, (2) stain poorly, (3) present in low numbers, (4) noncultivable, or (5) exhibit an atypical morphology.

3. In early infections by HSV-1 and HSV-2, the strongest immunohistochemical expression of HSV is observed within the epidermal keratinocytes, while in early infections caused by VZV, the strongest immunohistochemical staining is seen in the cells of the outer root sheath of the hair follicle and in sebocytes of the sebaceous gland.

4. identifying Epstein-Barr–encoded RNAs (EBERs) by ISH has a higher sensitivity and specificity than immunohistochemical detection using commercialized antibodies against EBV latent membrane protein (LMP)-1.

5. Immunohistochemical staining with anti-CMV monoclonal antibody gives a nuclear staining pattern within the endothelial cells of the dermis in early-stage infections, and a nuclear and cytoplasmic pattern in late-stage infections.

6. The immunohistochemical detection of HHV-8 in the nuclei of proliferating cells in KS lesions can be demonstrated in all its epidemiologic variants and from the earliest phases of the process.

7. Immunohistochemical detection of the HPV L1 capsid antigen is indicative of replicative HPV infections, but this method does not facilitate identification of the virus type involved, thus indicating that IHC is only of limited value in the management of HPV infections.

8. Immunohistochemical detection of PVB19 in the cytoplasm of endothelial cells of congested capillaries of the papillary dermis in skin lesions of PPGSS and infectious erythema is pathogenically significant when the biopsy specimens are performed in the clinical setting of PPGSS and other related exanthems.

9. Immunoexpression of the monoclonal antibody SV-40 in lesional skin of patients with TS demonstrates positive staining of the large eosinophilic cellular inclusions within keratinocytes composing the inner root sheath of hair follicles.

10. In patients with measles, immunoexpression of the monoclonal antibody is found in the nuclei of epidermal keratinocytes, keratinocytes of the hair follicle, and lymphocytes of the dermal infiltrate.

11. Immunoexpression for VP1 peptide of the enterovirus group is seen in epidermal keratinocytes and acrosyringeal keratinocytes of patients with HFMD.

12. Immunoexpression for HTLV-1 is seen as a dot-like paranuclear positivity, probably located in Golgi apparatus of neoplastic lymphocytes, in patients with ATTL.

13. Due to overpurification by the manufacturer in recent years, B124 antibody can no longer be used for screening of bacterial and fungal infections, as originally proposed, and the current potential use of the commercially available anti-BCG antibody should be restricted to the search of mycobacteria in FFPE samples.

14. Due to cross-reactions, the anti-*Mycobacterium tuberculosis* antibody can be used in the immunohistochemical diagnosis of cutaneous tuberculosis, leprosy and several cutaneous infections due to atypical mycobacteria.

15. IHC demonstrates different patterns of *T. pallidum* distribution in the skin depending on the stage of the infection: a “vasculotropic pattern” and “mixed vasculotropic-epitheliotropic pattern in observed in primary syphilis, while an “epitheliotropic pattern” in secondary syphilis.

16. We could immunohistochemically demonstrate the presence of *C. trachomatis* within the cytoplasm of histiocytes in the granulomatous areas of lesional skin in patients with LGV. These detection was performed using a monoclonal antibody reactive against D/K and L2 serovars of the bacteria. These findings have not been previously reported in the literature.

16. There are commercially available antibodies against *B. henselae* and *B. quintana* and, therefore, distinction between the two can be immunohistochemically performed in lesional tissue.

17. Different reports have demonstrated immunohistochemical detection of microcolonies of *N. meningitidis* within leukocytes, small blood vessels, and the dermal interstitium in skin biopsy specimens of patients with purpura fulminans. These authors used different non-commercially available monoclonal antibodies against *N. meningitidis*, however we failed to reproduce their findings using the monoclonal antibody that is commercially available.

Conclusiones

1. Un total de 25 anticuerpos disponibles comercialmente fueron identificados y aplicados a muestras de piel provenientes de pacientes con un amplio abanico de enfermedades infecciosas por virus y bacterias.

2. La IHQ resultó ser especialmente útil en la identificación de microorganismos que: (1) son difíciles de detectar con las tinciones de rutina o especiales, (2) se tiñen con dificultad, (3) están presentes en bajo número en la piel, (4) no son cultivables o crecen con dificultad en los cultivos, y (5) presentan una morfología atípica.

3. En las infecciones tempranas por virus herpes simple (VHS)-1 y 2 la intensidad de la tinción IHQ es mayor en los queratinocitos de la epidermis, mientras que en infecciones tempranas causadas por el virus varicela-zoster (VVZ) la mayor intensidad se observa en la vaina radicular externa del folículo piloso y los sebocitos de la glándula sebácea.

4. La identificación de ARNs codificados del virus de Epstein-Barr mediante hibridación in situ (HIS) tiene una mayor sensibilidad y especificidad que la detección IHQ del virus usando anticuerpos disponibles comercialmente frente a la proteína latente de mebrana-1 del virus.

5. El patrón de tinción IHQ del citomegalovirus en la piel es principalmente nuclear en las fases tempranas de la infección, y citoplasmático en fases más avanzadas.

6. La presencia de material genómico del HHV-8 en los núcleos de las células

proliferantes del sarcoma de Kaposi puede demostrarse mediante IHQ en todas sus variantes epidemiológicas y desde las fases más iniciales del proceso.

7. La detección IHQ del antígeno de cápside L1 del virus del papiloma humano (VPH) indica replicación activa del virus, pero no permite identificar el tipo de virus implicado en la infección cutánea, por lo que la utilidad de la IHQ en las infecciones por VPH es limitada.

8. La detección IHQ de PVB19 en el citoplasma de las células endoteliales de los capilares congestivos de la dermis papilar en muestras cutáneas de síndrome papular purpúrico en “guante y calcetín” (SPPGC) y eritema infeccioso, tiene relevancia diagnóstica en biopsias cutáneas realizadas en el contexto clínico de SPPGC.

9. La detección IHQ del anticuerpo monoclonal SV-40 en biopsias cutáneas de pacientes con tricodisplasia espinulosa muestra positividad en las inclusiones nucleares eosinofílicas de los queratinocitos de la vaina radicular interna de los folículos pilosos.

10. La detección IHQ del virus del sarampión en la piel demuestra presencia viral en los núcleos de los queratinocitos epidérmicos y del folículo piloso, así como en los linfocitos del infiltrado inflamatorio dérmico.

11. La detección IHQ del péptido VP1 común al grupo de los enterovirus demuestra presencia viral en los núcleos de los queratinocitos epidérmicos y del acrosiringio en muestras de piel de pacientes con enfermedad boca-mano-pie.

12. La detección IHQ del virus HTLV-1 demuestra presencia viral en la región paranuclear (aparato de Golgi) de los linfocitos neoplásicos en pacientes con Leucemia /linfoma de células T del adulto.

13. Debido a sucesivas purificaciones llevadas a cabo por el fabricante, el anticuerpo B124 no es útil en el diagnóstico de screening de infecciones víricas y bacterianas como se propuso originalmente, y su uso, hoy en día, se limita a la detección de infecciones por micobacterias.

14. Debido a reacciones cruzadas con otras bacterias, el anticuerpo anti-*Mycobacterium tuberculosis* es útil en el diagnóstico de tuberculosis, lepra y algunas infecciones cutáneas por micobacterias atípicas.

15. La detección IHQ de *T. pallidum* en biopsias de piel de pacientes con sífilis pone de manifiesto diferentes patrones de distribución del microorganismo según el estadío de la infección, con un patrón "vasculotrópico" y mixto "vasculotrópico-epidermotrópico" en sífilis primaria y un patrón "epidermotrópico" en sífilis secundaria.

16. La detección inmunohistoquímica de *C. trachomatis* con un anticuerpo monoclonal dirigido contra los serotipos D/K and L2 de la bacteria, demonstró la presencia de microorganismos en el citoplasma de los histiocitos en las áreas granulomatosas de pacientes con linfogranuloma venéreo. Estos hallazgos no han sido descritos previamente en la literatura.

16. Existen anticuerpos monoclonales específicos para la detección de *B. henselae* y *B. quintana* y, por tanto, la distinción entre ambas puede hacerse mediante IHQ en muestras de tejido fijado en parafina.

17. Diferentes autores han demostrado la presencia de microcolonias de *N. meningitidis* en los leucocitos, capilares y en el intersticio de la dermis en biopsias de pacientes con púrpura fulminans. Estos autores usaron anticuerpos monoclonales no

disponibles comercialmente, sin embargo, nosotros non hemos logrado confirmar estos hallazgos utilizando el anticuerpo monoclonal que está disponible comercialmente para la detección de *N. meningitidis*.

Curriculum Vitae summary

Ana María Molina Ruiz, MD

PERSONAL DATA

Dermatology specialist and Assistant proffesor at Fundación Jiménez Díaz, Universidad Autónoma de Madrid, Madrid, Spain.

Languages

Mother tongue: Spanish.

English:

09/1997
Level 12, Graded Examinations in Spoken English (GESE). Trinity College, London.

12/200
Cambridge Certificate of Proficiency in English (CPE). University of Cambridge.

09/1994-06/1999
Spanish Official School of Languages, Graduation in English.

09/1999-06/2000
English for Medical Doctors, University of Zaragoza, Spain.

French:

09/1994-06-2001: Spanish Official School of Languages, Graduation in French.

Education, training and professional experience

09/1998-06/2004
Medical Licensure. University of Zaragoza Medical School, Spain, MD.

05/2005-05/2009
Residency, Department of Dermatology, Virgen del Rocio University Hospitals, Sevilla, Spain.

01/2009-06/2009
Dermatology specialist at Dermagroup Dermatology Clinic, Sevilla, Spain.

09/2009-11/2009
Dermatology specialist at Hospital Sanitas La Moraleja, Madrid, Spain.

07/2009-11/2009
Dermatology specialist at Hospital Clínico San Carlos, Madrid, Spain.

11-2009-present
Dermatology specialist at Fundación Jiménez Díaz, Madrid, Spain.

01-2013-present
Coordinator of the Multidisciplinary Unit of Pigmentary Lesions and Melanoma at Fundación Jiménez Díaz, Madrid, Spain.

09-2014-present
Assistant professor of Dermatology. Universidad Autónoma de Madrid, Madrid, Spain.

International experience

07/2001-09/2001
Internship at the Department of Paediatric Surgery, Lastenklinikka Hospital, Helsinki, Finland.

11/2008-02/2009
International Observership program in Dermatology and Dermatopathology. University of California, San Francisco (UCSF), USA.

07/2014-09/2014
International Fellowship program in Dermatology and Dermatopathology at Memorial Sloan Kettering Cancer Center. New York, USA.

10/2014-01/2014
International Fellowship program in Dermatopathology at Dermatopathologische Gemeinschaftspraxis Friedrichshafen, Germany.

Awards and scholarships

05/1998
First Award in the National Pre-University Exam (Premio Extraordinario de Bachillerato).

1999, 200, 2001
Scholarships of the National Medical Student Association (IFMSA):
- Department of Internal Medicine, Hospital Virgen de la Victoria, Málaga, Spain.
- Department of Cardiology, University Hospital Morales Meseguer, Murcia, Spain.

09/2001-09/2002
Séneca Scholarship of the Spanish Ministry of Education and Science (MEC), University of Granada, Spain.

09/2003-05/2004
University of Zaragoza Research Program Scholarship. Department of Toxicology and Legal Medicine, University of Zaragoza, Spain.

09/2006
First award for Best Oral Presentation: “Primary Cutaneous Plasmacytoma”. I National Congress for Residents of Dermatology, Tenerife, Spain.

09/2007
First award for Best Oral Presentation: “Cytomegalovirus infection manifesting as distal ischemia of the lower limb”). II National Congress for residents of Dermatology, Madrid, Spain.

2007-2009

European Academy of Dermatology and Venereology (EADV) Scholarships to participate in:

- 1st Summer School of Sexually Transmitted Diseases, Sofia, Bulgaria.
- 1st and 2nd Summer Schools of Demopathology", Graz, Austria.
- 4th European Training Programme Euroderm Excellence, Rome, Italy.
- Summer School on Cutaneous Lymphomas", Copenhague, Denmark.
- Summer school on Autoimmunity and Psoriasis, Berlin, Germany.
- Summer School of Microbiology". Vienna, Austria.
-"John Sratigos Memorial Scholarship" at the XVII European Congress of Dermatology and Venereology, Paris.

10/2010

First award for Best Oral Presentation, ("Saurian Papulosis"). Reunion of the Spanish Academy of Dermatology, Madrid, Spain.

07/2014-01/2015

International Research Scholarship for Medical professionals. Fundación Conchita Rábago.

Coordination positions and representation activities

09/1998-09/2004

Spanish Coordinator of the International Federation of Medical Student Association (IFMSA)

09/2007-09/2009

Member of the Board of Directors of the regional section of Médicos del Mundo, Andalucía, Spain.

09/2006-06/2009

National Representative of Spanish Dermatology Residents in the Spanish Academy of Dermatology and Venereology (AEDV).

09/2007-06/2009

Editor of the First Dermatology Manual for the Spanish Residents of Dermatology.

10/2008

Organization of the III National Congress of the Spanish Dermatology Residents. Spanish Academy of Dermatology and Venereology, Sevilla, Spain.

01/2010-present
Member of the editorial Board of Actas Dermo-sifiliográficas.

01/2013-present
Coordinator of the Spanish Research on Trichology Group.

06/2014-present
Coordinator of the Innovation and New Technologies Group of the Spanish Academy of Dermatology and Venereology.

Publications

Articles in scientific journals

1. Barabash R, Moreno-Suárez FG, Rodríguez L, **Molina AM**, Conejo-Mir J. Nelson syndrome: a rare cause of generalized hyperpigmentation of the skin. Actas Dermo-Sifiliográficas. 2010;101:76-80.

2. Revelles JM, Machan S, Pielasinski U, Camacho D, **Molina A**, Requena L. Lipofribomatosis. Monografías de Dermatología. 2011;24:262-263.

3. Revelles JM, Machan S, Pielasinski U, Camacho D, **Molina A**, Requena L. Tumor fibrolipomatoso hemosiderótico. Monografías de Dermatología. 2011;24:264-265.

4. Revelles JM, Machan S, Pielasinski U, Camacho D, **Molina A**, Requena L. Lipoblastoma y lipoblastomatosis. Monografías de Dermatología. 2011;24:266-269.

5. **Molina-Ruiz AM**, Molina-Ruiz RM, Zulueta T, Barabash R, Requena L. Olanzapine-induced eccrine squamous syringometaplasia. American Journal of Dermatopathology. 2012;34:434-7.

6. **Molina-Ruiz AM**, Luque R, Zulueta T, Bernabeu J, Requena L. Cytomegalovirus-induced cutaneous microangiopathy manifesting as lower limb ischemia in a human immunodeficiency virus-infected patient. Journal of Cutaneous Pathology. 2012;39:945-9.

7. **Molina-Ruiz AM**, Pulpillo A, Lasanta B, Zulueta T, Andrades R, Requena L. A rare case of primary cutaneous plasmacytoma-like lymphoproliferative disorder following renal transplantation. Journal of Cutaneous Pathology. 2012;39:685-9.

8. **Molina-Ruiz AM**, Requena L. Implantes cosméticos en Dermatología: características y efectos adversos. Medicina Cutánea Ibero Latino Americana. 2012; 40:131-146.

9. **Molina-Ruiz AM**, Bernabeu J. Disseminated leishmaniasis in a patient infected with human immunodeficiency virus. Piel 2012;27:164-6.

10. **Molina-Ruiz AM**, Pérez-Vega E, Zulueta T. Chronic plantar ulcer in an African inmigrant. Actas Dermo-Sifiliográficas. 2012;103:733-4.

11. **Molina-Ruiz AM**, del Carmen Fariña M, Carrasco L, Santonja C, Rodríguez-Peralto JL, Torrelo A, Kutzner H, Requena L. Saurian papulosis: a new clinicopathological entity. Journal of the American Academy of Dermatology. 2013;68:17-22.

12. **Molina-Ruiz AM**, Domine M, Requena L. Acute and severe acne in a patient treated with bevacizumab. International Journal of Dermatology. 2013;52:486-90.

13. **Molina-Ruiz AM**, Sanmartín O, Santonja C, Kutzner H, Requena L. Spring and summer eruption of the elbows: a peculiar localized variant of polymorphous light eruption. Journal of the American Academy of Dermatology. 2013;68:306-12.

14. **Molina-Ruiz AM**, Requena L. Reply to: Summertime elbow eruptions. Journal of the American Academy of Dermatology. 2013;69:485.

15. **Molina-Ruiz AM**, García-Gavín P, García-Gavín J, Boada-García A, Carrascosa-Carrillo JM. Actas 2.0: Actas Dermo-Sifiliográficas joins the world of social networking. Actas Dermo-Sifiliográficas. 2013;104:735-7.

16. **Molina-Ruiz AM**, Cerroni L, Kutzner H, Requena L. Cutaneous deposits. American Journal of Dermatopathology. 2014;36:1-48.

17. Vañó-Galván S, **Molina-Ruiz AM**, Serrano-Falcón C, Arias-Santiago S, Rodrigues-Barata AR, Garnacho-Saucedo G, Martorell-Calatayud A, Fernández-Crehuet P, Grimalt R, Aranegui B, Grillo E, Diaz-Ley B, Salido R, Pérez-Gala S, Serrano S, Moreno JC, Jaén P, Camacho FM. Frontal fibrosing alopecia: A multicenter review of 355 patients. Journal of the American Academy of Dermatology. 2014;70:670-8.

18. **Molina-Ruiz AM**, García-Gavín P, García-Gavín J, Boada-García A, Carrascosa-Carrillo JM. Actas Dermo-Sifiliográficas on Facebook. Actas Dermo-sifiliográficas. 2014 Sep;105(7):635-638.

19. Machan S, **Molina-Ruiz AM**, Fernández-Aceñero MJ, Encabo B, LeBoit P, Bastian B, Requena L. Nodular lesions arising in a giant congenital melanocytic nevus in an adult: a case report with molecular characterization. American Journal of Dermatopathology. 2014 Jul 24. [Epub ahead of print] PubMed PMID: 25062263.

20. **Molina-Ruiz AM**, Santonja C, Rütten A, Cerroni L, Kutzner H, Requena L. Immunohistochemistry of viral infections. Part I. Cutaneous viral infections by herpesviruses and papillomaviruses. American Journal of Dermatopathology. 2014 Aug 28. [Epub ahead of print] PubMed PMID: 25171431.

21. **Molina-Ruiz AM**, Santonja C, Rütten A, Cerroni L, Kutzner H, Requena L. Immunohistochemistry of viral infections. Part II. Cutaneous Viral Infections by Parvoviruses, Poxviruses, Paramyxoviridae, Picornaviridae, Retroviruses and Filoviruses. American Journal of Dermatopathology. 2014 Aug 28. [Epub ahead of print] PubMed PMID: 25171430.

22. Carranza C, **Molina-Ruiz AM**, Pérez de la Fuente T, Kutzner H, Requena L, Santonja C. Subungual acral fibromyxoma involving the bone: a mimicker of malignancy. American Journal of Dermatopathology. 2014 Jul 24. [Epub ahead of print] PubMed PMID:25062265.

23. **Molina-Ruiz AM**, Cerroni L, Kutzner H, Requena L. Immunohistochemistry in the diagnosis of cutaneous bacterial infections. American Journal of Dermatopathology. 2014. (In press).

24. **Molina-Ruiz AM,** Requena L. Cutaneous foreign body granulomas. Dermatologic Clinics. 2014. (In press)

25. **Molina-Ruiz AM,** Romero F, Carrasco L, Feltes F, Haro R, Requena L. Amyophatic dermatomyositis presenting as a flagellated skin eruption with positive MDA 5 antibodies and thyroid cancer association. International Journal of Dermatology. 2014. (In press)

26. Carranza C, **Molina-Ruiz AM,** Cuevas J, Requena L. Cutaneous epithelioid hemangioendothelioma on the sole of a child. Pediatric Dermatology. 2014. (In press)

27. Bernárdez C, Machan S**, Molina-Ruiz AM,** Pérez de la Fuente T, Pavón M, Carrillo I, Fortes J, Requena L. Dermatofibrosarcoma protuberans of the vulva with myoid differentiation. American Journal of Dermatopathology. 2014. (In press)

28. Bernárdez C, **Molina-Ruiz AM**, Requena L. Histopatología de las alopecias. Parte I. Alopecias no cicatriciales. Actas Dermo-Sifiliográficas. 2014. (In press).

29. Bernárdez C, **Molina-Ruiz AM**, Requena L. Histopatología de las alopecias. Parte II. Alopecias cicatriciales. Actas Dermo-Sifiliográficas. 2014. (In presss).

30. Pérez de la Fuente T, Cárcamo C, Requena L, **Molina-Ruiz AM**, Caballero O. Actitud quirúrgica en el angiosarcoma cutáneo de Wilson. Piel. 2014 (In press).

31. **Molina-Ruiz AM**, Ortiz-Reina S, Carranza C, Kutzner H, Requena L. Sebocyte-like cell primary cutaneous melanoma: a rare cytologic variant of malignant melanoma. American Journal of Dermatopathology. 2014 (In press).

32. Vañó-Galván S, **Molina-Ruiz AM**, Fernández-Crehuet P, Rodrigues-Barata R, Arias-Santiago S, Serrano-Falcón C, Martorell-Calatayud A, Barco D, Urech M, Alonso L, Grillo E, Pérez B, Jiménez-Gómez N, Garnacho-Saucedo G, Serrano S, Requena L, Grimalt R, Jaén P, Camacho F.M. Folliculitis decalvans: a multicenter review of 82 patients. Journal of the American Academy of Dermatology. 2014 (In press).

33. **Molina-Ruiz AM**, Bernárdez C, Arno Rütten, Luis Requena. Merkel cell carcinoma within poroma: report of two cases. J Cutan Pathol. 2014 (In press).

Articles in scientific divulgation journals

1. **Molina-Ruiz AM**. Médicos en Formación. Salud Global. El Dermatólogo. 2007;9:6-7.
2. **Molina-Ruiz AM**. EADV Summer School: training course on Dermatopathology. EADV News. 2007;24:16-17.
3. **Molina-Ruiz AM.** La importancia del cuidado de la piel joven. IM Farmacias. 2010;9:50-51.
4. **Molina-Ruiz AM**. Reuniones de Residentes de Dermatología. Dermactual. 2008;1:4.
5. **Molina-Ruiz AM**. La III Reunión Nacional de Residentes de Dermatología. Dermactual. 2008;2:8-9.
6. **Molina-Ruiz AM**. Presente y Futuro de los Residentes de Dermatología. Dermactual. 2009;7:21.
7. **Molina-Ruiz AM**. Fotoprotección solar de la piel en verano. Harper´s Bazaar. 2010;Junio:80-81.
8. **Molina-Ruiz AM**. Campaña Fotoprotégete bien. El Mundo. Mayo; 2012.
9. **Molina-Ruiz AM**. Cuidados de la piel en verano. Revista Día. Junio; 2010.
10. **Molina-Ruiz AM**. Entrevista a la Dra. Molina. Revista InStyle. Mayo; 2013.
11. **Molina-Ruiz AM**. Fotoprotección solar y Unidad de Lesiones pigmentarias. Revista Impulso FJD. Mayo; 2014.

Book chapters

1. **Molina-Ruiz AM**, Requena L. Lesiones histológicas elementales. En: Conejo-Mir et al. *Manual de Dermatología*. Editorial Aula Médica. 2010: 47-60. ISBN: 978-84-7885-490-5.

2. Conejo-Mir J, **Molina-Ruiz AM**. Carcinoma Basocelular. En: Conejo-Mir et al. *Manual de Dermatología*. J. Editorial Aula Médica. 2010:1165-1182. ISBN: 978-84-7885-490-5.

3. Pereyra-Rodríguez JJ, Gacto-Sánchez P, Bernabeu-Wittel J, **Molina-Ruiz A**, Domínguez-Cruz J, Pulpillo-Ruiz A, Conejo-Mir J. Estrategias de prevención de tumores cutáneos en receptores de órganos sólido. En: *Actualizaciones en trasplantes. Hospital Virgen del Rocío.* 2010. www.huvr.es/trasplantes.

4. Gavín J, **Molina-Ruiz AM**. Reglas nemotécnicas. En: Molina-Ruiz et al., eds. *Manual de Dermatología para Residentes.* Editorial Glosa. 2011:826-834. ISBN: 978-84-7429-522-1.

5. Pielasinsky U, **Molina-Ruiz AM**. Tratamiento médico en Dermatología. En: Molina-Ruiz et al., eds. *Manual de Dermatología para Residentes.* Editorial Glosa. 2011:777-791. ISBN: 978-84-7429-522-1.

6. **Molina-Ruiz AM**, Mazaira-Fernández M. Systemic Immunosupressants. En: Conde-Taboada, A. *Dermatologic Therapies*. 2012: 283-362. Editorial Bentham Science Publishers Ltd. United Arab Emirates. 2012. ISBN: 978-1-60805-431-2.

7. **Molina Ruiz A,** Requena L. Seudolinfomas cutáneos. En: *Dermatología Práctica Ibero-Latino-Americana*, 2ª Edición, Torres V, Camacho F, Mihm M, Gonzalez S, Jurado E, Sánchez Carpintero E, eds. México, Lito Laser SA de CV, 2012: 137.1-123.12.

8. **Molina-Ruiz AM**. Alopecia androgenética e hiperplasia benigna de próstata. En: Arias- Santiago S, Serrano S. *Comorbilidades en Alopecia Androgenética*. Glosa. (In press)

9. Machan S, **Molina-Ruiz AM**, Requena L. Dermal Hypertrophies. En: Bolognia JL, Schaffer JV, Cerroni L. *Dermatology*, Fourth edition, London, Elsevier. (In press).

10. **Molina-Ruiz AM**, Fuertes L, Carranza C, Requena L. Immunohistology and Molecular Studies of Sweat Gland Tumors. En: Plaza J. *Applied Immunohistochemistry and Molecular Studies in the Evaluation of Skin Neoplasms*. Springer Science + Business Media. (In press)

11. **Molina-Ruiz AM,** Requena L. Panniculitis. En: Jeffrey P. Callen, Joseph L. Jorizzo. Dermatological Signs of Systemic Disease. 5th Edition. Elsevier. (In press).

Teaching activities/invited lectures

1. **"Sexually Transmitted Diseases".** Center on International Cooperation, Médicos del Mundo Andalucía, Sevilla, Spain, 22 September 2007.

2. **"Residency training in Dermatology".** II National Congress of the Spanish Dermatology Residents, Madrid, 26-27 October 2007.

3. **"Basic curriculum in Psychodermatology".** National Reunion of the Spanish Dermatology and Psychiatry Group". Zaragoza, Spain, 22-23 February 2008.

4. **"Psychodermatology, a new subspecialty in Dermatology".** 36th National Congress of the Spanish Academy of Dermatology and Venereology. Barcelona, Spain, June 2008.

5. **"Training perspectives and future proffesional activities of a Dermatology Resident".** Residerm, Barcelona, Spain, January 2009.

6. **"Management strategies of non-melanoma skin cancer in transplant patients".** 12th International Congress on skin cancer. Tel Aviv, Israel. 3-6 May, 2009.

7. **"Introduction to Psoriasis"**. I National Course of Psoriasis for Dermatology Residents, Valencia, Spain, 22-23 May 2009.

8. **"Quality of life of patients with psoriasis".** Update on Psoriasis Course, Barceloneta, Puerto Rico, 14-17 November 2009.

9. **"Cutaneous Exanthems"**. Emergency Dermatology Course. La Moraleja Hospital, Madrid, Spain, 18 December 2009.

10. **"Effects of tumor necrosis factor blockade on cardiovascular risk factors in patients with psoriasis".** 5th Annual Conference of Alexandria Society of Dermatology, Venereology and Andrology, Alexandria, Egypt, 18-20 February 2010.

11. **"Management strategies for non-melanoma skin cancers in transplant patients".** The Egyptian-Spanish Dermatology Conference. Alexandria, Egypt, 18-20 February 2010.

12. **"Vasculitis".** III Course of Emergency Dermatology. University Hospital Gregorio Marañón, Madrid, Spain, 19 November 2010.

13. **"Emergency scenarios in Dermatology".** V National Congress of the Spanish Dermatology Residents. Málaga, Spain, 16-17 September 2011.

14. **"Actas Dermo-Sifiligráficas joins the world of social networking".** 40th National Congress of the Spanish Academy of Dermatology and Venereology. Oviedo, Spain, 6-9 June 2012.

15. **"Immunohistochemistry of viral infections".** XIX Congreso Ibero-Latinoamericano de Dermatología (CILAD). Sevilla, Spain, 18-22 September 2012.

16. **"Complex clinical cases in trichology".** I Update on Trichology Course. University Hospital Ramón y Cajal, Madrid, Spain, 17 May 2013.

17. **"Androgenetic alopecia".** Alopecias Update Course. Hotel Room Mate Oscar, Madrid, Spain, 22 May 2013.

18. **"Saved by the patient".** 17th Joint Meeting of the International Society of Dermatopathology-American Society of Dermatopathology. Denver, USA. 19-20 March 2014.

19. **"Research in Trichology".** II Update on Trichology Course University Hospital Ramón y Cajal, Madrid, Spain, 28 March 2014.

20. **"Histopathology of Alopecias".** Alopecias Update Course. Hotel Loft, Madrid, Spain. 8 May 2014.

21. **"Immunohistochemistry in the diagnosis of cutaneous infections".** Memorial Sloan Kettering Cancer Center, 28th July 2014.

22. **"Immunohistochemistry relevant to Dermatopathology".** Memorial Sloan Kettering Cancer Center, 1st August 20014.

Oral presentations

76 Oral Communications in National and International Congresses

Courses, congresses and reunions attended

15 International Courses attended
17 International Reunions and Congresses attended
90 National Courses attended
30 National Reunions and Congresses attended

Extracurricular activities and other employment experience

International Cooperation

09/1994-10/1998: Coordination of the Red Cross “young volunteer” program, Zaragoza, Spain.

06/2003-08/2003: Medical staff volunteer at Red Cross Inmigration Center, Tarifa, Cádiz, Spain.

05/2006-05/2009: Médicos del Mundo. Homeless program, Sevilla, Spain.

05/2007-05/2009: Médicos del Mundo. Coordination of the regional HIV program, Sevilla, Spain.

08/2007-09/2007: Medical staff volunteer at Mother Theresa of Kolkata Center, Kolkata, India.

07/2008-09/2008: Médicos del Mundo, Pharmacy system analysis program in the districts of Quissanga, Ibo, Mecufi and Metuge in the province of Cabo Delgado, Mozambique, Africa.

Camp America International Exchange Program.

06/1999-09/1999: Medical Staff, Camp Skimino, Virginia. USA.

06/2000-09/2000: Medical Staff, Camp Na-Sho-Pa, New York. USA.

06/2001-09/2001: Medical Staff, Camp Valley-Forge, Philadelphia. USA.

06/2002-09/2002: Medical Staff, Camp Blue-Ridge, Pennsylvania. USA.

01/2000-05/2009: Interviewer at Camp America Recruitment Center, London, United Kingdom.

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