**Objectives:** To characterize human immunodeficiency virus (HIV)–associated granuloma annulare (GA) by clinical, microscopic, and molecular methods and to investigate the role of Epstein-Barr virus infection in the pathogenesis of GA.

**Design:** Patients were evaluated clinically, and biopsy specimens of lesional skin were examined by light microscopy. Polymerase chain reaction and in situ hybridization for Epstein-Barr virus were performed on 4 and 12 biopsy specimens, respectively.

**Setting:** Academic referral center.

**Patients:** Thirty-four consecutive HIV-positive patients who have a clinical and histological diagnosis of GA.

**Outcome Measures:** Clinical distribution of lesions, light-microscopic features, and the presence of Epstein-Barr virus DNA and RNA in biopsy specimens.

**Results:** Granuloma annulare was generalized in 20 patients and localized in 14. Twenty patients (59%) presented with acquired immunodeficiency syndrome. Unusual features were the presence of oral lesions in 1 patient, perforating lesions in 2 patients, and the coexistence of GA and Kaposi sarcoma in 1 biopsy specimen. Microscopic examination of 34 biopsy specimens showed a granulomatous pattern that was interstitial in 8, palisaded in 18, perforating in 2, and mixed interstitial and palisaded in 6. Special staining of all specimens was negative for organisms. Epstein-Barr virus infection was not detected by either polymerase chain reaction or in situ hybridization.

**Conclusions:** Generalized GA is the most common clinical pattern in HIV infection. Granuloma annulare associated with HIV can present at all stages of HIV infection, but it is slightly more common in patients with acquired immunodeficiency syndrome. Epstein-Barr virus is an unlikely causative agent of HIV-associated GA. Granuloma annulare may be a manifestation of increasing immune dysregulation.

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**CUTANEOUS manifestations of human immunodeficiency virus (HIV) infection are diverse and include neoplasms, infections, and inflammatory conditions.**

In 1985, Penneys and Hicks reported the cases of 2 patients with the acquired immunodeficiency syndrome (AIDS) who presented with a disseminated papular eruption resembling granuloma annulare (GA). Since then, 21 other cases of GA in HIV-infected patients have been reported in the English-language literature. A report raised questions regarding the relation of HIV-associated GA and Epstein-Barr virus (EBV) infection. Generalized GA has been reported to be more prevalent than localized GA in HIV-infected patients. Because 34 patients with concurrent GA and HIV disease have been seen at our institution in 11 years, we undertook this study to characterize HIV-associated GA by clinical, microscopic, and molecular methods and to investigate the role of EBV infection in the pathogenesis of GA.

**RESULTS**

**CLINICAL FEATURES**

Of the 34 patients, 32 were male; the patients ranged in age from 20 to 61 years. Twenty-seven were white, 4 were African American, and 3 were Hispanic. The mean age at the onset of GA was 38.9 years. At the onset of GA, 20 patients (59%) met the Centers for Disease Control and Prevention criteria for AIDS. Twenty-four
PATIENTS AND METHODS

The diagnosis of GA was made from biopsy specimens of 34 HIV-infected patients seen from July 1984 to July 1995. The distribution of skin lesions of GA was classified as either localized or generalized on clinical grounds. Cases of GA were classified as localized if they were solitary or few and appeared on only 1 area of the body and generalized if many lesions were present on the trunk and upper or lower extremities. One patient who had AIDS and oral mucosal GA was previously described by Green et al. None of the patients had diabetes mellitus. Sections from 34 biopsy specimens from patients with HIV-associated GA were reviewed by us (J.R.T., P.C., and P.E.L.) for the presence of mucin, degenerated collagen, inflammatory infiltrate, small vessel leukocytoclastic vasculitis, pauci-inflammatory thrombosis, and transepidermal elimination of collagen.

We also classified cases by the pattern of inflammatory skin disease, as described by Ackerman: interstitial, palisaded, and mixed interstitial and palisaded granulomas. Cases were classified as interstitial GA if histiocytes and a sparse lymphocytic infiltrate were present in a perivascular and interstitial pattern. They were classified as palisaded GA if histiocytes were present in a palisaded pattern around foci of necrobiosis with mucin deposition. They were classified as mixed interstitial and palisaded GA if histiocytes and a sparse lymphocytic infiltrate were present in an interstitial and palisaded pattern around foci of degenerated collagen with mucin deposition. Vasculitis was defined as the presence of fibrin in venular walls or lumens, in the context of an inflammatory cell infiltrate in or around vessel walls. Neutrophils, lymphocytes, eosinophils, or histiocytes may infiltrate the vessel wall, depending on the diagnosis. Colloidal iron or Alcian blue stains were used to detect acid mucopolysaccharides. Acid-fast, periodic acid–Schiff, Gomori methenamine-silver, and Warthin-Starry silver stains were used for the detection of organisms.

EBV POLYMERASE CHAIN REACTION

DNA was extracted from formalin-fixed and paraffin-embedded tissue from 4 recent biopsy specimens from HIV-positive patients with GA. Polymerase chain reaction was performed as described previously for the presence of EBV DNA. Briefly, thick sections were cut from each block, deparaffinized, dried, and boiled in buffer (100 µL). The presence of amplifiable DNA was verified by the ability to detect human β-globin gene DNA by polymerase chain reaction. The primers used for EBV amplification are from the EBV NA1 gene and span the first 78 nucleotides of the gene. Their sequences are 5'-AGTCTGACGAGGGCCAGGTACA and 5'-GGAGCTTCTGGTCCAGATGTGTC. For each amplification, 20 µL of crude DNA from paraffin blocks was used in a total reaction volume of 50 µL. Amplification profile consisted of 40 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute. The products of amplification were electrophoresed on a 5% agarose gel (NuSieve; FMC Corporation, Rockland, Me) and visualized with ethidium bromide staining under UV light. DNA from Raji cells was amplified in each reaction and served as a positive control.

EBV IN SITU HYBRIDIZATION

The RNA in situ hybridization technique has been described previously. Briefly, 5-µm-thick sections of paraffin-embedded tissue were prepared on silanated slides. Tissue sections were deparaffinized, rehydrated, permeabilized with Triton X-100, and digested with proteinase K (10 µg/mL). Riboprobes were applied in a 50% formaldehyde hybridization buffer, and the slides were hybridized overnight. Following posthybridization washes, antidigoxigenin–alkaline phosphatase antibody conjugate was applied to each slide. The slides were then washed and placed into a color-developing solution consisting of nitroblue tetrazolium and X-phosphate. The reaction was stopped by washing the slides in an appropriate buffer. The slides were counterstained with eosin, and coverslips were applied.

The integrity of the RNA in each tissue section was evaluated with a digoxigenin-labeled riboprobe directed at an abundant cellular RNA polymerase II transcript, U6. Sections showing hybridization signal with the U6 probe were determined adequate for analysis with the EBER1 probe. The EBV EBER1 riboprobe was prepared as previously described. Slides prepared from a paraffin-embedded tissue block containing metastatic nasopharyngeal carcinoma to lymph node were used as positive controls.

patients presented with multiple discrete skin-colored dermal papules, and 2 patients had nodules. The remaining patients had annular dermal plaques. The predominant location of GA was the upper extremities, followed by the trunk. One patient had GA of the oral mucosa in addition to skin lesions. Twenty patients (59%) had generalized (Figure 1) and 14 (41%) had localized lesions. Two patients with localized GA had perforating lesions. Two patients denied exacerbation by UV-light exposure, and lesions were not photodistributed.

HISTOLOGICAL FEATURES

Microscopic evaluation revealed normal epidermis except 2 cases showed a transepidermal elimination of collagen. There was focal degenerated collagen with macrophages and lymphocytes in an interstitial pattern in 7 biopsy specimens, a palisaded pattern in 16, and a mixed interstitial and palisaded pattern in 3. Two biopsy specimens showed a palisaded pattern with transepidermal elimination or perforation of connective tissue. One showed degenerated collagen pinching into a follicular orifice with a central parakeratotic plug, and another showed palisading histiocytes extending to an atrophic epidermis underlying compact parakeratosis (Figure 2). None had a pattern of deep or subcutaneous GA. None of the specimens showed granulomatous or leukocytoclastic vasculitis, pauci-inflammatory thrombosis, or interstitial neutrophilic infiltrates. All biopsy specimens showed numerous lymphocytes and macrophages. Multinucleated macrophages were prominent in 7 specimens. Mast cells were noted in 5 specimens and eosinophils in 2. Plasma cells were noted in 2 specimens, 1 of oral mucosa.
One biopsy specimen showed both Kaposi sarcoma (KS) and interstitial GA. Polarized light-microscopic examination revealed no foreign bodies. Biopsy specimens showed mucin deposition within areas of collagen degeneration between collagen bundles by hematoxylin and eosin stain. Six specimens were also stained with colloidal iron and 2 with Alcian blue to confirm increased dermal mucin deposition. Special stains, such as the Fite method for acid-fast bacilli (23 specimens), periodic acid–Schiff (7 specimens), Gomori methenamine-silver (15 specimens), and Warthin-Starry silver (14 specimens), were all negative for organisms.

MOLECULAR STUDIES

Sufficient intact DNA was present in 4 biopsy specimens of HIV-associated GA, as shown by a sharp band with β-globulin amplification (data not shown). Polymerase chain reaction of Raji cells used as a positive control showed a specific band of the expected size (78 base pairs) (data not shown). This band, however, was absent in all 4 patients with HIV-associated GA (data not shown). Adequate tissue RNA was present in all 12 patients tested, as shown by strong in situ hybridization signals with the U6 riboprobe. Epstein-Barr viral RNA was not detected in any of the 12 patients with HIV-associated GA (Figure 3). However, EBV RNA was detected in sections from metastatic nasopharyngeal carcinoma of lymph node used as positive controls.

COMMENT

Granuloma annulare is a chronic cutaneous eruption that presents clinically as dermal papules, plaques, or nodules. Lesions can be localized, perforating, generalized, erythematous, or subcutaneous. Diabetes mellitus, iritis, sarcoidosis, autoimmune thyroiditis, and various neoplasms have been found in association with GA.23 No case of diabetes mellitus, however, has been reported in association with GA and HIV infection. Twenty-three cases of GA in patients with HIV infection have previously been reported.4-17 This study presents 34 additional cases of histologically confirmed GA in HIV-infected patients and 2 cases of perforating GA associated with HIV infection.

Clinically, our patients’ lesions consisted mostly of discrete papules similar to those described by Dabski and Winkelmann.24 We found that HIV-associated GA is more commonly generalized than localized (20 of our patients [59%]). Also, 15 (65%) of 23 previously reported cases of HIV-associated GA were generalized.3-17 Thus, the incidence of generalized GA appears to be increased in HIV infection compared with sporadic GA and GA in association with other systemic diseases. Because GA lesions are mostly asymptomatic or associated only with mild pruritus, we think that cases are underreported.

The histological findings in our patients are similar to those in immunocompetent patients with GA, with the exception of a biopsy specimen that showed both KS and GA. In HIV-positive patients, KS has been concurrently present in lesions of other neoplasms20 and infections.27

Interstitial GA and KS can be easily confused microscopically because they both can have an increased number of ovoid or spindle cells situated between collagen bundles in the reticular dermis, but they have different architectural and cytologic components. The increase in the number of endothelial cells is most prominent around adnexal structures in KS but not in GA. Although slitlike vascular spaces between reticular dermal collagen bundles and around preexisting vascular and adnexal structures are seen in KS, they are absent in GA. Interstitial mucin and mast cells are commonly seen in GA but not in KS. Plasma cells are a common perivascular infiltrate in KS, but they are rare in GA. The differential diagnosis of HIV-associated GA includes a variety of infections, granulomatous lymphoproliferative processes, granulomatous drug reactions, and lichenoid and granulomatous dermatitis of AIDS (LGDA). Granuloma annulare has been described in association with various infectious stimuli, the spectrum of which encompasses Borrelia species, mycobacteria, fungus, HIV, EBV, and hepatitis C.1-17,26-31 and hence, it is difficult to exclude that the granulomatous response is related to microbial pathogens other than HIV. Syphilis, mycobacteria, and fungal infection appear unlikely causes of HIV-associated GA. Histological features associated with infection such as tubercles of epithelioid histiocytes, foamy histiocytes, plasma cells, caseation necrosis, and neutrophilic abscesses were absent in all of our biopsy specimens. In addition, Warthin-Starry silver, Fite, and Gomori methenamine-silver stains failed to reveal spirochetes, mycobacteria, or spores and hyphae, respectively.

Figure 1. Numerous skin-colored dermal papules distributed over the trunk and extremities.
Postviral granulomatous reactions similar to GA have been described due to herpes simplex and varicella zoster.28-32 Granulomas are usually limited to the location of the previous viral infection. Although granulomatous reactions are thought to be induced by residual viral proteins, DNA sequences have been found31 in lesional skin. We do not believe that HIV-associated GA represents a postherpetic viral granulomatous reaction because none of our patients had clinical evidence or a history of herpes simplex or varicella-zoster viral infection.

In 1988, Spencer et al17 reported a GA-like eruption in a patient with chronic EBV infection who presented with annular lesions on the face and extensor aspects of the arms and legs and elevated titers to several EBV antigens in serum specimens. The described histological findings, however, were different from that of GA because neutrophils were present, and both degeneration of collagen and mucin were absent. In this study, polymerase chain reaction for the EBV genome (n = 4) and in situ hybridization for EBV RNA (n = 12) were performed in specimens with retrievable DNA and RNA, but no evidence of EBV infection was found. In addition, our patients did not show systemic signs of EBV infections such as oral hairy leukoplakia or clinical signs of mononucleosis. Therefore, EBV is unlikely to be the cause of our patients' skin eruptions.

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Lymphoproliferative disorders that can present with granulomatous reactions include granulomatous mycosis fungoides, granulomatous slack skin, and angiocentric lymphoma—including lymphomatoid granulomatosis, natural killer/T-cell lymphoma, and angiocentric T-cell lymphoma. Granuloma annulare associated with HIV can be differentiated histologically from granulomatous slack skin and mycosis fungoides by architectural and cytologic features. It lacks the marked epidermotropism and the bandlike lymphocytic infiltrates in a fibrotic papillary dermis that may be present in granulomatous mycosis fungoides; also, the large giant cells found in granulomatous slack skin do not occur in HIV-associated GA. Atypical lymphocytes do not infiltrate the wall of vessels as they do in the angiocentric lymphomas.33

The differential diagnosis of HIV-associated GA also includes 2 newly described conditions, interstitial granulomatous drug reaction (IGDR)34 and LGDA.35,36 Granuloma annulare associated with HIV lacks the distinctive clinical presentation of IGDR, which consists of violaceous plaques localized to intertriginous zones, medial thighs, and inner arms. Histologically, both GA and IGDR can show an interstitial granulomatous infiltrate; however, a vacuolar interface reaction and atypical lymphocytes along the dermoepidermal junction with variable epidermotropism, as have been reported in IGDR, are absent in HIV-associated GA. Granuloma annulare associated with HIV and LGDA share some clinical and histological attributes. The distribution of disseminated GA and LGDA can be identical. Papules of LGDA, however,
have a lichenoid character with a superficial scale, which is usually lacking in HIV-associated GA. On histological examination, both HIV-associated GA and LGDA can show clusters of macrophages, but LGDA has a lichenoid lymphocytic infiltrate and lacks mucin deposition within granulomatous foci. The presence of abundant dermal mucin in areas not infiltrated by histiocytes is a non-specific finding in many skin lesions in patients with HIV infection and does not differentiate between HIV-associated GA and LGDA.

Although the cause of GA is still unknown, UV light, insect bites, trauma, and neoplasia have been proposed as precipitating factors. 3-7 To our knowledge, only 3 cases of photosensitive GA with HIV infection have been reported9-11,37, 2 with generalized lesions and 1 localized to the feet. Two patients with generalized GA had lesions in sun-exposed areas, and phototesting revealed sensitivity to UV-B light. 9,11,37 Histological features characteristic of photodermitis of HIV, 38 such as necrotic keratinocytes, spongiosis, exocytosis, and vacuolar or lichenoid interface changes, were absent in biopsy specimens from our patients. In addition, our patients said that their eruption was not exacerbated by UV-light exposure, and the lesions were not photodistributed. We did not do phototesting of our patients, however. Vasculitis has been reported11 in some cases of GA in immunocompetent patients. Recently, Magro et al10 reported 4 cases of HIV infection associated with GA. They showed the presence of granulomatous vasculitis, leukocytoclastic vasculitis, and “thrombogenic vasculopathy” in biopsy specimens with GA-like histological features. In addition, they reported the presence of extravascular neutrophilia in 1 specimen. None of the patients in their series had a clinical diagnosis of GA. The clinical impressions were herpes zoster, eosinophilic folliculitis vs keratosis pilaris, and dermatitis herpetiformis. Because these patients did not have a detailed clinical description and had extravascular neutrophilia, they may represent a spectrum of granulomatous reaction patterns that share some histological features with GA. In contrast, in previous reports8-13,17 and in our series of cases of HIV-associated GA, similar findings could not be identified in biopsy specimens that histologically showed GA.

Granuloma annulare is a relatively common eruption in HIV-infected patients. Generalized GA is more commonly observed in HIV-infected patients, compared with GA observed in individuals not infected with HIV. The histological findings of HIV-associated GA are similar to those seen in non–HIV-infected persons. Finally, GA does not seem to be directly related to EBV infection. Because HIV-infected patients commonly suffer from fungal, mycobacterial, and viral infections that can present similar to GA both clinically and histologically, we recommend a biopsy of lesional skin to exclude an infectious cause.

CONCLUSIONS

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