Clin Exp Immunol 1994; 97:260–265

Immunohistological assessment of cutaneous drug hypersensitivity in patients with HIV infection

A. CARR*, E. VASAK*, V. MUNRO†, R. PENNY* & D. A. COOPER*‡ *Centre for Immunology and †Department of Anatomical Pathology, St. Vincent's Hospital, and the ‡National Centre in HIV Epidemiology and Clinical Research, University of New South Wales, Sydney, Australia

(Accepted for publication 5 May 1994)

SUMMARY

The pathogenesis of drug hypersensitivity in patients with HIV infection is unknown. To study further the nature of hypersensitivity, the histopathological features of morbilliform drug hypersensitivity reactions were examined in a group of HIV-infected patients. Skin sections from 23 HIV-infected subjects with morbilliform drug hypersensitivity reactions were examined by light microscopy, direct immunofluorescence and immunohistochemistry, to determine the nature of the inflammatory infiltrate and the role of immunoglobulin, complement and cytokines. The principal light microscopic findings were spongiosis, hydropic generation of the basal layer, civatte bodies, an epidermal lymphocytic infiltrate (48%), and a perivascular dermal infiltrate of lymphocytes (87%) and macrophages (52%). Two patients had findings consistent with toxic epidermal necrolysis. Immunohistochemistry demonstrated that the lymphocytic infiltrate consisted of $CD8^+$, $HLA\text{-}DR^+$ T lymphocytes (some of which also stained for CD38), a marked depletion of epidermal Langerhans cells (90%), and strong cytoplasmic staining of keratinocytes for IL-6 (60%), IL-1β (50%), tumour necrosis factor-alpha (TNF-α) (45%) and to a lesser degree, interferon-gamma (IFN-γ) (35%). Immunofluorescence did not demonstrate any significant deposition of immunoglobulin or complement. The histological findings were independent of the responsible drug, the duration of either therapy or the rash, and of peripheral blood $CD4^+$ and $CD8^+$ cell counts. These findings suggest that activated $CD8^+$ lymphocytes and perhaps epidermal production of cytokines are involved in the pathogenesis of cutaneous drug hypersensitivity in HIV-infected patients. The common histological features, regardless of the causative drug, suggest a common pathogenesis.

Keywords drug hypersensitivity cytokines human immunodeficiency virus

INTRODUCTION

Drug hypersensitivity reactions occur commonly in patients with HIV disease. Hypersensitivity manifests as a morbilliform, cutaneous eruption, often preceded by fever, after 8–14 days therapy [1,2]. Features of IgE-mediated hypersensitivity have not been observed. Hypersensitivity is significantly more common in patients with HIV infection than in those with other immunodeficiency disorders or in the general population [3–7]. The causes and mechanisms of this hypersensitivity are not known, but are likely to include a high dose or prolonged duration of therapy, the degree of immunodeficiency, a slow acetylation phenotype, glutathione deficiency, and perhaps coexisting viral infection or immune complex deposition [8–10]. Hypersensitivity to trimethoprim-sulphamethoxazole (TMP-SMX) in HIV-infected and HIV-uninfected patients has been attributed to toxic hydroxylamine drug metabolites that are inactivated by glutathione [11,12]. The association of fever with cutaneous hypersensitivity suggests that inflammatory cytokines, such as IL-1, IL-6, tumour necrosis factor-alpha (TNF-α) or interferon-gamma (IFN-γ), may be involved in its pathogenesis.

In a previous study, light microscopic assessment of morbilliform cutaneous drug hypersensitivity reactions in a group of HIV-infected subjects found most to have a lymphocytic infiltrate [13]. However, no immunofluorescence or immunohistochemistry was performed. In the present study, immunofluorescence and immunohistochemistry were performed to help elucidate the pathogenesis of drug hypersensitivity, and in particular to examine the nature of any inflammatory infiltrate, as well as the roles of immunoglobulin, complement and cytokines.

Correspondence: Dr Andrew Carr, Centre for Immunology, St. Vincent's Hospital, Sydney, Australia 2010.

SUBJECTS AND METHODS

Subjects

Samples were obtained from 23 HIV-infected subjects who had developed typical morbilliform reactions following institution of amoxicillin ($n=4$), sulphadiazine ($n=4$), dapsone ($n=4$), nevirapine ($n=4$), TMP-SMX ($n=2$), and one each of carbamazepine, clindamycin, cephalothin, pentamidine and rifampicin. Control samples were obtained from three HIV-infected subjects who were receiving TMP-SMX with no manifestations of hypersensitivity and who had macroscopically normal skin.

Clinical data

The following data were collected at the time of biopsy: causative drug; indication for therapy; dose and duration of therapy at the time of biopsy; duration and features of hypersensitivity; T lymphocyte subset values before commencement of therapy. Each hypersensitivity reaction was defined as previously [14]. Briefly, hypersensitivity was defined by the development of a characteristic, morbilliform, cutaneous eruption that occurred after institution of therapy and resolved upon drug discontinuation. Causality was only attributed either where no other drug commonly associated with hypersensitivity (e.g. other antibiotics, anticonvulsants, opiates) had been commenced concurrently, or if the hypersensitivity resolved despite continuation of these other drugs.

Collection of specimens

Specimens were collected from patients after obtaining written, informed consent. Skin biopsies were performed following local anaesthesia with lignocaine 1% (without adrenaline), and were obtained from either the torso, upper arm or anterior thigh. Samples consisted of 2×4 mm punch biopsies, which were either fixed in formalin and embedded in paraffin for light microscopy or frozen in OCT compound (Miles Diagnostics, Elkhart, IN) and stored at $-70°C$ for subsequent direct immunofluorescence or immunohistochemistry.

Light microscopy

Paraffin-embedded tissue was cut at 6 μm thickness, and sections were stained with haematoxylin and eosin.

Immunofluorescence

FITC-conjugated polyclonal rabbit antibodies to the following were used for direct immunofluorescence: IgG, IgM, IgA, C3, C1q and fibrinogen (all from Dako, Carpenteria, CA). Following sectioning and acetone fixation, slides were washed in buffer (0·1 M Tris phosphate pH 7·6), incubated for 30 min with FITC-conjugated antibody, rewashed with buffer and then mounted after application of buffered glycerol.

Immunohistochemistry

Antibodies to the following cell surface proteins or cytokines were used: CD3 (UCHT1; Dako), CD45 (LC; Dako), CD4 (OKT4; Orthodiagnostics, Raritan, NJ), CD8 (OKT8; Orthodiagnostics), CD20 (L26; Dako), CD1 (T6; Coulter Immunology, Hialeah, FL), CD14 (Leu-M3; Becton Dickinson, Mountain View, CA), CD25 (Becton Dickinson), CD38 (Leu-17; Becton Dickinson), HLA-DR (Becton Dickinson), TNF-α (Genzyme, Boston, MA), IL-1β (Genzyme), IL-6 (Genzyme), and IFN-γ (Endogen, MA). Following sectioning, mounting and acetone fixation, slides were washed in buffer (0·1 M Tris phosphate pH 7·6), blocked with normal goat serum (5 min) and incubated with primary antibody at appropriate dilutions for 30 min at room temperature, except for cytokine antibodies, which were incubated overnight at 4°C. After further washing, endogenous peroxidase was blocked with 3% hydrogen peroxide (5 min). Slides were rewashed with buffer between subsequent incubations with biotinylated goat anti-mouse IgG/goat anti-rabbit IgG (10 min), streptavidin/horseradish peroxidase (10 min), and diaminobenzidene (1 mg/ml)/0·02% hydrogen peroxide (4 min) (Dako LSAB Kit, Dako). Slides were then counterstained with methylene blue and haematoxylin before dehydration, clearing and mounting.

RESULTS

Clinical features

All 23 subjects were homosexual men with symptomatic HIV disease. Their median $CD4^+$ cell count at the time of hypersensitivity was 44 (range 0–390) $\times 10^6/l$, the median $CD8^+$ cell count was 460 (range 141–1804) $\times 10^6/l$, and the median CD4:CD8 ratio was 0·09 (range 0–0·41). Their median age was 35 years. The cardinal feature in each subject was a widespread morbilliform rash; two patients had exfoliation or mucosal involvement. One subject receiving TMP-SMX had concurrent transient hypoplastic bone marrow (confirmed histologically) and renal failure. One patient receiving dapsone also had severe haemolytic anaemia. No patient had eosinophilia. Of the 23 subjects, 19 ceased therapy as a result of hypersensitivity. Two subjects were receiving corticosteroids at the time of biopsy (prednisolone 50 mg daily, as adjunctive therapy to TMP-SMX for *Pneumocystis carinii* pneumonia (PCP) in one subject, and as therapy for the rash in the other subject). The median duration of therapy at commencement of the reactions was 10 (range 1–32) days, and at the time of biopsy was 12 (range 2–42) days. Of the 23 rashes, nine were biopsied within 24 h of onset, nine within 3 days, three at days 4 or 5, and two were biopsied at day 10. The control subjects all had advanced, symptomatic HIV disease.

Light microscopy

Typical light microscopic findings are seen in Fig. 1. Sections showed spongiosis, hydropic degeneration of the basal layer, civatte bodies, and occasional hyperkeratosis and parakeratosis. In 20 (87%) of 23 biopsies, there was a predominantly perivascular dermal infiltrate of lymphocytes, described as mild in 12 subjects and moderate in eight subjects. In addition, small numbers of dermal macrophages were seen in 12 biopsies, all of which had a dermal lymphocytic infiltrate. Three biopsies showed a mild eosinophilic infiltrate. There was a mild to moderate epidermal lymphocytic infiltrate in nine (39%) biopsies. Two patients had more extensive findings consistent with toxic epidermal necrolysis, with widespread keratinocyte necrosis and a more intense epidermal and dermal infiltrate, including epidermal macrophages. Three biopsies showed no cellular infiltrate.

Immunofluorescence

Only three of 20 biopsies from the HIV-infected subjects demonstrated any positive fluorescence. One biopsy showed sparse granular IgG at the dermo-epidermal junction, another

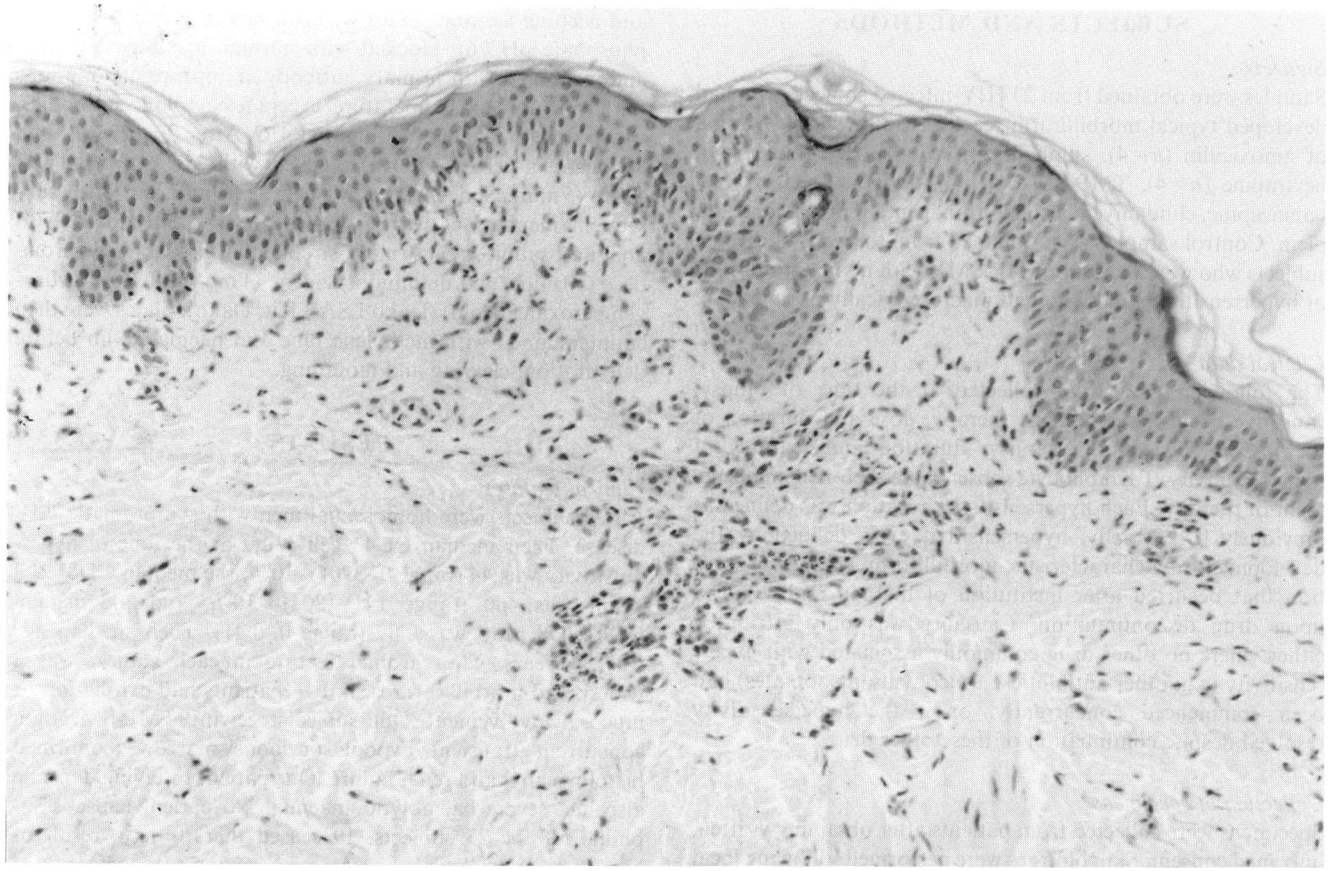

Fig. 1. Light microscopy of a typical hypersensitivity reaction in an HIV-infected subject. There is a lymphocytic infiltrate in the dermis and epidermis, with scattered dermal macrophages, and epidermal spongiosis, civatte bodies and hydropic degeneration of the basal layer (×100).

showed similar staining for IgA, and the third showed granular C3 deposits in relation to the endothelium of a few mid-dermal vessels.

Immunohistochemistry

Immunohistochemistry was performed on biopsies from 20 HIV-infected subjects (Fig. 2). The lymphocytic infiltrate was composed of $CD8^+$ T lymphocytes ($CD45^+$, $CD3^+$, $CD8^+$, $CD4^-$, $CD20^-$). $CD8^+$ cells from each biopsy were $HLA\text{-}DR^+$ and occasionally $CD38^+$ (25%), but only one biopsy showed any lymphocytic staining for IL-2R. Keratinocytes were also positive for HLA-DR in 75%. No B lymphocytes ($CD45^+$, $CD3^-$, $CD20^+$) were seen. The presence of dermal and epidermal monocytic cells ($CD45^-$, $CD14^+$, $CD4^+$, $CD3^-$, $CD8^-$) was confirmed.

There was a marked depletion of epidermal Langerhans cells. Only two (10%) of 20 biopsies showed epidermal cellular staining for CD1. A further five biopsies showed focal weak intercellular CD1 epidermal staining.

There was a moderate to strong cytoplasmic staining of keratinocytes for IL-6 (60%), IL-1β (50%), TNF-α (45%) and IFN-γ (35%). There was focal, upper dermal interstitial staining for several cytokines, consistent with diffusion of these cytokines into the dermis from the epidermis. There was positive staining of the cytoplasm of some dermal lymphocytes, macrophages or epithelia of sebaceous glands, sweat glands and/or hair follicles and occasional endothelial cells for IL-6 (33%), IL-1β (25%), TNF-α (25%) and IFN-γ (20%).

Control skin from the HIV-infected subjects showed subjective reduction in the numbers of Langerhans cells, and no cytokine staining of keratinocytes.

Relationship of histological findings to clinical and laboratory variables

No correlation was found between the light microscopic findings and peripheral blood T cell subsets, the causative drug, the duration of rash, the duration of drug therapy, or the degree of positive cytokine staining. In particular, there was no correlation found between the presence or degree of $CD8^+$ lymphocytes infiltrating the skin and the peripheral blood $CD8^+$ lymphocyte count or the duration of the rash. Similarly, there was no correlation found between the immunohistochemical findings and any other variable.

DISCUSSION

Several features of cutaneous hypersensitivity reactions in HIV-infected patients are apparent from the above data. First, the light microscopic features are similar, if not identical, to clinically identical reactions in HIV-uninfected patients [15],

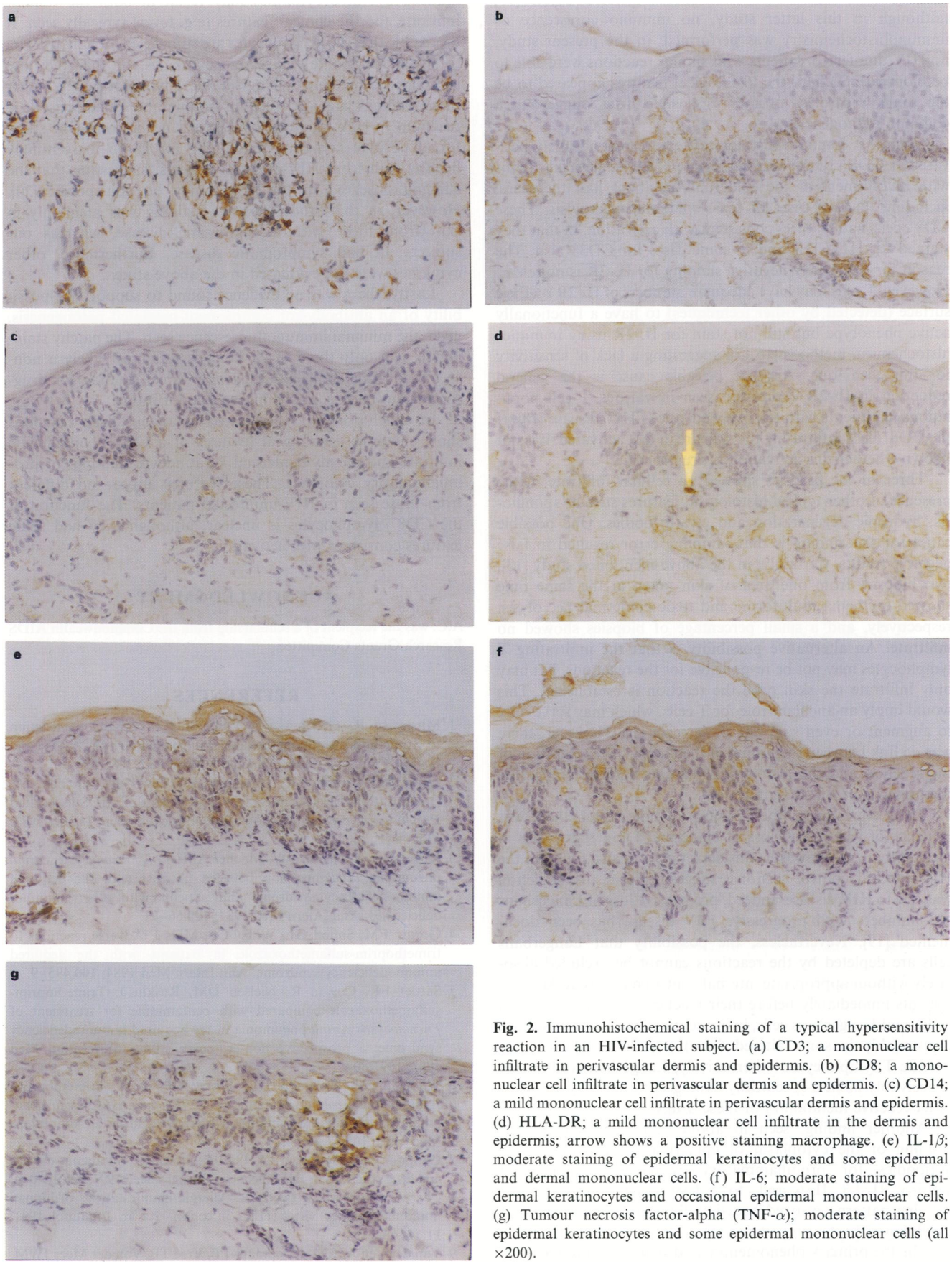

Fig. 2. Immunohistochemical staining of a typical hypersensitivity reaction in an HIV-infected subject. (a) CD3; a mononuclear cell infiltrate in perivascular dermis and epidermis. (b) CD8; a mononuclear cell infiltrate in perivascular dermis and epidermis. (c) CD14; a mild mononuclear cell infiltrate in perivascular dermis and epidermis. (d) HLA-DR; a mild mononuclear cell infiltrate in the dermis and epidermis; arrow shows a positive staining macrophage. (e) IL-1β; moderate staining of epidermal keratinocytes and some epidermal and dermal mononuclear cells. (f) IL-6; moderate staining of epidermal keratinocytes and occasional epidermal mononuclear cells. (g) Tumour necrosis factor-alpha (TNF-α); moderate staining of epidermal keratinocytes and some epidermal mononuclear cells (all $\times$200).

as well as those reported in a series of HIV-infected subjects [13] (although in this latter study, no immunofluorescence or immunohistochemistry was performed; in the present study, no HIV-uninfected patients with similar reactions were able to be biopsied). Second, the histological features appeared to be the same, regardless of the responsible drug, suggesting a common pathogenesis.

The most common histological feature was a largely perivascular $CD8^+$ lymphocytic infiltrate of the superficial dermis with involvement of the epidermis, in about half the cases, including the two cases of toxic epidermal necrolysis. These $CD8^+$ cells had features consistent with activation, in that they stained for HLA-DR and in some cases for CD38 also. The reason for the largely negative staining for IL-2R is not clear. However, T cells may have adequate numbers of IL-2R on their surface (detected by other techniques) to have a functionally active phenotype but still not stain for IL-2R using immunohistochemical methods [16,17], suggesting a lack of sensitivity for this technique. Another possible cause is the general reduction in IL-2R expression seen in various T cell populations in patients with relatively advanced HIV disease [18]. If the $CD8^+$ cells infiltrating the skin are in fact activated, it is not known whether this activation is drug-specific.

Three of the biopsies showed no cellular infiltrate in the presence of other typical histological features such as spongiosis, hydropic degeneration and civatte bodies. One possible cause for this finding is that sampling error resulted in false negative results. Certainly, in the above-mentioned study [13], two biopsies from one area of skin taken at the same time showed erythema multiforme and toxic epidermal necrolysis, respectively, and a small percentage of biopsies showed no infiltrate. An alternative possibility is that the infiltrating T lymphocytes may not be responsible for the reactions, but may only infiltrate the skin once the reaction is established. This would imply an ancillary role for T cells, which may serve only to augment or even suppress these reactions. However, there was no link between the timing of the biopsy or the duration of the reaction and the histological features detected (including the presence of a T cell infiltrate).

There were three other findings of note. First, there was evidence of widespread loss of epidermal Langerhans cells in most specimens. However, this depletion was also found in the HIV-infected control sections, suggesting that the depletion was due to HIV disease. Indeed, progressive loss of Langerhans cell numbers with progressive HIV disease has been documented [19]. Nevertheless, the possibility that Langerhans cells are depleted by the reactions cannot be excluded absolutely without appropriate internal control biopsies of the same patients immediately before their reactions. It is possible that epidermal Langerhans cells, by presenting drug on their surface (presumably in some altered form), are damaged by drug-specific T lymphocytes. Alternatively, Langerhans cells could conceivably be non-specifically damaged by hydroxylamine or other reactive metabolites of these drugs.

Another finding, seen in about half the biopsies, was staining of the cytoplasm of epidermal keratinocytes with antibodies for proinflammatory cytokines, in particular TNF-α, IL-1β, IL-6 and, to a lesser extent, IFN-γ. This staining pattern occurred occasionally even in the absence of a cellular infiltrate, suggesting that this production of cytokines may be the primary phenomenon, and that local inflammation induced by these cytokines may result in both the cellular infiltrate and the clinical features (e.g. fever) typically seen. It is possible that the keratinocytes are stimulated directly by drug (or drug metabolite) to secrete cytokines, or that they are stimulated by activated, drug-specific T lymphocytes. Whether these findings are also seen in comparable cutaneous drug reactions in HIV-uninfected subjects is not known. One study found positive keratinocyte staining for IL-1β in seemingly healthy skin from HIV-infected subjects, suggesting that the staining observed in this study may be non-specific [20]. However, in that study, positive staining was seen only in skin from those with asymptomatic disease, whereas our subjects all had symptomatic disease. Furthermore, other cytokines were not evaluated in the above study.

Lastly, there was no evidence found to support the possibility of an antibody- or complement-mediated pathogenesis, given the minimal immunofluorescence seen. The patchy staining seen in only three of 20 samples was most likely a non-specific phenomenon, although only direct staining for drug-specific antibody could definitively confirm this.

In conclusion, HIV-associated cutaneous drug reactions show common histological features of a $CD8^+$ lymphocytic infiltrate and mostly epidermal keratinocyte staining for pro-inflammatory cytokines. These features appear no different from those seen in HIV-uninfected patients. The function of the $CD8^+$ lymphocytes is unclear, although they had some features consistent with activation.

ACKNOWLEDGMENT

A.C. was the recipient of a scholarship from the Commonwealth AIDS Research Grants Committee.

REFERENCES

1 Mitsuyasu R, Groopman J, Volberding P. Cutaneous reaction to trimethoprim-sulfamethoxazole in patients with AIDS and Kaposi's sarcoma (letter). N Engl J Med 1983; **308**:1535–6.

2 Jaffe HS, Abrams DI, Ammann AJ, Lewis BJ, Golden JA. Complications of co-trimoxazole in treatment of AIDS-associated *Pneumocystis carinii* pneumonia in homosexual men. Lancet 1983; i:1109–11.

3 Kovacs JA, Hiementz JW, Macher AM *et al. Pneumocystis carinii* pneumonia: a comparison between patients with the acquired immunodeficiency syndrome and patients with other immunodeficiencies. Ann Intern Med 1984; **100**:66–71.

4 Gordin FM, Simon GL, Wofsy CB, Mills J. Adverse reactions to trimethoprim-sulfamethoxazole in patients with the acquired immunodeficiency syndrome. Ann Intern Med 1984; **100**:495–9.

5 Sattler FR, Cowan R, Nielsen DM, Ruskin J. Trimethoprim-sulfamethoxazole compared with pentamidine for treatment of *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome: a prospective non-crossover study. Ann Intern Med 1988; **109**:280–7.

6 Wharton JM, Coleman DL, Wofsy CB *et al.* Trimethoprim-sulphamethoxazole or pentamidine for *Pneumocystis carinii* pneumonia in the acquired immunodeficiency syndrome. Ann Intern Med 1986; **105**:37–44.

7 Coopman MA, Johnson RA, Platt R, Stern RS. Cutaneous disease and drug reactions in HIV infection. N Engl J Med 1993; **328**:1670–4.

8 Carr A, Cooper DA, Penny R. Allergic manifestations of human immunodeficiency virus (HIV) infection. J Clin Immunol 1991; **11**:55–64.

9 Van der Ven AJAM, Koopmans PP, Vree TB, Van der Meer JWM.

Adverse reactions to co-trimoxazole in HIV infection. Lancet 1991; **338**:431–3.

10 Carr A, Gross A, Hoskins J, Penny R, Cooper DA. Acetylation phenotype and hypersensitivity to trimethoprim-sulphamethoxazole in HIV-infected patients. AIDS 1994; **8**:333–7.

11 Rieder MJ, Uetrecht J, Shear NH, Cannon M, Miller M, Spielberg SP. Diagnosis of sulfonamide hypersensitivity reactions by *in vitro* 'rechallenge' with hydroxylamine metabolites. Ann Intern Med 1989; **110**:286–9.

12 Carr A, Tindall B, Penny R, Cooper DA. *In vitro* cytotoxicity as a marker of hypersensitivity to trimethoprim-sulphamethoxazole in HIV-infected patients. Clin Exp Immunol 1993; **94**:21–25.

13 Matis WL, Triana A, Shapiro R, Eldred L, Polk BF, Hood AF. Dermatologic findings associated with human immunodeficiency virus infection. J Am Acad Dermatol 1987; **17**:746–51.

14 Carr A, Swanson C, Penny R, Cooper DA. Clinical and laboratory markers of hypersensitivity to trimethoprim-sulfamethoxazole in patients with *Pneumocystis carinii* pneumonia and AIDS. J Infect Dis 1993; **167**:180–5.

15 Elias PM, Fritsch PO. Erythema multiforme and toxic epidermal necrolysis. In: Dermatology and general medicine. Fitzpatrick TB, Eisen AZ, Wolff K *et al.*, eds. New York: McGraw-Hill, 1987:555–67.

16 Zola H, Mantzioris BX, Webster J, Kette FE. Detection by immunofluourescence of surface molecules present in low copy numbers: high sensitivity staining and calibration of flow cytometer. J Immunol Methods 1990; **135**:247–55.

17 Zola H, Neoh SH, Mantzioris BX, Webster J, Loughnan MS. Circulating human T and B lymphocytes express the p55 interleukin receptor molecule (TAC, CD25). Immunol Cell Biol 1989; **67**:233–7.

18 Fauci AS. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science 1988; **239**:617–22.

19 Dreno B, Milpied B, Bignon JD, Stalder JF, Litoux P. Prognostic value of Langerhans cells in the epidermis of HIV patients. Br J Dermatol 1988; **118**:481–6.

20 Dreno B, Milpied B, Dutarte H, Litoux P. Epidermal interleukin 1 in normal skin of patients with HIV infection. Br J Dermatol 1990; **123**:487–92.