Characterization of Mononuclear Cell Infiltrates in Psoriatic Lesions

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The dermal mononuclear cell infiltrates of psoriatic lesions were characterized by receptors for sheep erythrocytes (T-lymphocytes), C3b receptors (macrophages and B-lymphocytes) and C3d receptors (B-lymphocytes), using hemadsorption to cryostat sections in a closed chamber. T-lymphocytes and macrophages were the predominant inflammatory cells. Very few B-lymphocytes were detected. These findings are discussed in relation to the possible pathogenetic significance of cellular immunity in psoriasis.

In recent years evidence points to the significance of humoral immunity in the pathogenesis of psoriasis [1–11]. Whether cell-mediated immunity also may be of pathogenetic significance remains to be clarified. The results reported have been conflicting [12–17], and studies on T-lymphocytes in psoriatic lesions have been hampered by lack of sufficiently sensitive methods for their demonstration in tissue sections in situ [18].

In this study we have succeeded in delineating T-lymphocytes in tissue sections using hemadsorption with sheep erythrocytes treated with 2-aminoethylisothiouronium bromide hydrobromide (AET) (Sigma, St. Louis, Mo., U.S.A.) Similarly, macrophages and B-lymphocytes were characterized by their known differences of cell surface receptors [19,20].

MATERIALS AND METHODS

Patients

Fourteen patients with untreated active fully developed psoriasis vulgaris and 5 patients with incipient lesions of pin-head size (1-2 mm in diameter) were studied.

Tissues

Skin biopsies from psoriatic lesions and unaffected areas of the same patient together with skin from unaffected normals were taken under local anesthesia. The specimens were quick-frozen in pre-cooled isopentane, embedded in Tissue-Tek II O.C.T. compound (Lab-Tek Products, Naperville, Ill., U.S.A.) and sectioned on a cryostat. Sections from all frozen specimens were stained with hematoxylin and eosin.

Specimens of normal spleen and kidney were obtained from the Department of Surgery.

Erythrocytes

Human blood and blood from sheep was collected in equal volumes of Aselver's solution and stored at 4°C. Before use, the erythrocytes were washed 3 times in 10 volumes of phosphate-buffered saline (PBS), pH 7.2 and packed at 1000 × g for 10 min.

Sera

Fresh normal human sera from blood donors were used as the source of complement (C). C was inactivated by heating at 56°C for 30 min. Inactivated sera absorbed with E were used as C3b inactivator reagent [21].

Rabbit IgM antibody (A) to E was isolated from early immune response antisera by gel filtration on Sephadex G 200 equilibrated with PBS.

Hemadsorption with Tissue Sections

The method was the same as described by Tønder, Milgrom, and Witebsky [22], using a closed chamber technique. Cryostat sections, 4–6 μ thick, of frozen tissue on large cover-glasses were incubated with the indicator cells applied to the sections using microculture slides with a single concavity. The concavity was filled with the indicator cells and sealed by the cover-glass with the tissue section submerged in the cell suspension. The slides were inverted and incubated in a wet chamber at varying temperatures and for varying lengths of time (see below). The slides were then turned, cover-slip up, and left so that detachment from the glass and nonreactive tissue could occur. The preparations were examined microscopically when all erythrocytes had detached from the glass.

Demonstration of E Receptors

E were treated with AET according to the procedure described by Kaplan and Clark [23]. The indicator cells (E_{AET}) were used as a 1% suspension in PBS containing 25% fetal bovine serum, stored at 4°C and used within 5 days. After incubation of the sections with indicator cells overnight at 4°C, the slides were turned and read microscopically while still cold.

Demonstration of Complement Receptors

The indicator cells were prepared as previously described [24]. E were sensitized with 1/2 agglutinating unit of A (IgMEA). One agglutinating unit is defined as the amount of the highest dilution of A which agglutinates an equal amount of 1% suspension of E. The washed IgMEA were made up to a 1% suspension in barbital (Veronal)-buffered saline, pH 7.2, containing 0.15 μM CaCl₂, 0.5 mM MgCl₂ and 0.01% gelatin (GVB). Zymosan-treated fresh human serum which retains considerable C3 activity with low C5 activity [25], were used as source of C. Equal volumes of 2% IgMEA and C diluted 1 in 8 in GVB were mixed and incubated for 10 min at 37°C. The cells were washed and made up to a 1% suspension in GVB (IgMEA C3b). Cell-bound complement is cleaved by C5 inactivator in serum to a fluid phase fragment C5c, and the cell-bound component C5b remains attached [26]. To split cell-bound C3b, 1 ml of a 1% suspension of IgMEAC3b in GVB was mixed with 2 ml of C3b inactivator reagent. The mixture was incubated at 37°C for 2–4 hr and finally made up to a 1% suspension in GVB (IgMEAC3d).

After incubation of the sections with the indicator cells for 30 min at room temperature, the slides were turned and read microscopically.

Controls

The reactivity of the E_{AET} was tested with tissue sections of specimens from human spleen, and the IgMEAC with tissue sections from both spleen and kidney. The E_{AET} indicator cells preferentially adhere to the periarteriolar sheaths in the white pulp of spleen [27] (Fig. 1). The IgMEAC3b adhere both within the white and the red pulp, and the IgMEAC3d to the white pulp areas only [27]. Glomeruli in human renal tissue have receptors for C3b only [24]. Immune adherence tests in tubes using 1% suspension of human O Rh-negative erythrocytes served as an additional control of reactivity of the two IgMEAC test
systems. IgMEAC3b induces immune adherence of primate erythrocytes, while IgMEAC3d does not [19,26]. The IgMEAC3d were not used in tests with tissue sections unless they were nonreactive in the immune adherence test.

Tests with IgMEA and IgMEA incubated with inactivated zymosan-treated human sera served as the control for nonspecific binding of indicator cells in tests using tissue sections.

**Fixation and Staining**

After microscopical reading, the preparations were fixed according to the procedure previously described with slight modification [24]. In tests with $E_{AET}$, when the slides were submerged in the fixatives, the coverglasses were carefully moved to one side allowing a small rim of opening between the edge of the coverglass and the concavity.

In order to enhance the contrast between the indicator cells and the tissue, some fixed sections were stained for 20 min at room temperature in Graham and Karnovsky's solution [28]. The sections were then gently washed for 15 min in PBS, stained with hematoxylin and eosin and mounted on slides.

**RESULTS**

$E_{AET}$ adhered exclusively to the dermal mononuclear cell infiltrates in sections from all fully active lesions as well as from initial pin-head sized lesions (Fig 2-4). The density of the dermal mononuclear cell infiltrates varied in the different lesions studied. However, irrespectively of the density of the infiltrates, the majority of the cells in the each infiltrate was covered by $E$, mostly as single cells, but rosettes were also observed (Fig 5). The rosettes were present only in native preparations, never after fixation and staining. No attachment of indicator cells was noted in sections from nonlesional or normal skin and none of the indicator cells adhered to the epidermis.

**DISCUSSION**

The IgMEAC3b indicator cells also adhered to the same dermal infiltrates (Fig 6). Comparing the results of the $E_{AET}$ and IgMEAC3b test, significantly fewer of the latter indicator cells attached. In some of the most active lesions, however, an equally strong adherence of $E_{AET}$ and IgMEAC3b was recorded. Very few of the IgMEAC3d indicator cells adhered to the infiltrates. None of the indicator cells adhered to normal skin.
cytes and macrophages. However, the dominance of T-lymphocytes seems to be greater than would be expected from the relation between T- and B-lymphocytes in peripheral blood where the ratio is about 4:1 [13,14]. Jondal [31] has found that B blast cells lack receptors for C3 and Fc part of the IgG molecule and argue that caution must be taken when these receptors are used as markers for B-lymphocytes. Since the method we have used, does not enable us to differentiate between B-lymphocytes and possible B blast cells, there may be cells present of B cell origin that we do not detect. Finally,

into the adjacent tissue. This may in turn explain the reduced number of T-lymphocytes in peripheral blood from patients during active stages of psoriasis as reported by others [12–14].

Using histochemical techniques, Braun-Falco and Schmoeckel [30] found a striking infiltrate of macrophages and lymphocytes in the dermis already in pinpoint sized psoriatic lesions in contrast to the relatively slight epidermal changes noted at this stage of events. These data together with the results in the present study lend support to the concept that the prime event of the psoriatic lesions may be mediated by cellular immunity [12].

The method presented is semiquantitative, and does not permit quantitating relative percentages of T- and B-lympho-

FIG 5. Unfixed and unstained preparation shows adherence of E₄₅ showing T-lymphocytes (benzidine H+E, mostly as single cells, but rosettes (arrows) are also present (reduced X Thickness from × 280).

FIG 6. Adherence of IgMEAC₃b indicating macrophages and B-lymphocytes. Section of the same lesion as in Fig 2–5 (benzidine and H+E, × 280).
the question has to be considered whether lymphoid cells, possibly a T-lymphocyte subpopulation, actually "home" in the skin as proposed by others [32,33]. We have examined dermal infiltrates of other dermatoses, i.e. lichen planus, systemic and discoid lupus erythematosus, and mycosis fungoides and constantly find a great dominance of T-lymphocytes [29,34]. Accordingly, at present we cannot exclude that the accumulation of T-lymphocytes in psoriatic lesions is nonspecific and of no pathogenetic significance. Further studies on these problems are in progress.

The authors thankfully acknowledge the skilled technical assistance of Mrs. I. Grimelund.

REFERENCES


