Lymphocyte Chemoattractants in Psoriasis and Normal Skin

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The local production of lymphocyte attractants may influence both physiologic lymphocyte trafficking in the skin as well as the infiltration of these cells in pathologic states. Recent evidence for the production of acidic lipid lymphocyte chemoattractants, particularly 12[R]-hydroxyeicosatetraenoic acid, in psoriatic lesions is reviewed. Water extractable lymphocyte attractant activity may also be recovered from both normal skin samples and psoriatic lesional stratum corneum, and may be important in the pathophysiology of lymphocyte trafficking. Less than 10 kD activity from normal skin has undergone the most detailed characterization. This has led to the isolation of a novel, as yet unidentified compound from normal skin, which we have termed “plasma-associated lymphocyte chemoattractant” (PALC). J Invest Dermatol 95:225–235, 1990

The primary pathogenic event in psoriasis is unknown, although some evidence suggests that vascular changes and leukocyte infiltration precede the keratinocyte proliferation that characterizes this disease. The nature of the initial leukocyte infiltrate seen in early developing lesions is also the subject of controversy, with different groups presenting evidence that either neutrophils, T-lymphocytes, or monocyte/macrophages may be the initial infiltrating cell.

Since the reports of the presence of substantial T-cell infiltrates in psoriatic lesions and of the therapeutic effectiveness of cyclosporine in psoriasis, interest in the role of lymphocytes in the pathogenesis of this disease has increased. We have, therefore, attempted systematic screening of lesional extracts for compounds that stimulate lymphocyte migration, on the basis of the hypothesis that the majority of lymphocytes in lesions are present as a result of increased migration from blood vessels rather than because of local proliferation.

ASSAY OF LYMPHOCYTE MIGRATION

The development and validation of an in vitro bioassay of lymphocyte migration of sufficient sensitivity and convenience to cope with the relatively small samples obtainable from human skin in vivo, and to allow screening of large numbers of chromatography fractions in several dilutions, have been described in detail [1]. Briefly, heparinized venous blood is purified by density gradient centrifugation and a two-stage plastic adherence step to yield mixed lymphocytes containing less than 1% monocytes. This level of cellular purification was required to prevent preferential migration of monocytes. The lymphocytes (50 μl of a 2 × 10⁶ ml⁻¹ suspension) are placed in the upper wells of a 48-well microchemotaxis apparatus (Neuro Probe, Cabin John, MD) and separated from test material (25 μl) in the lower wells by an 8-μm pore-size polycarbonate membrane. Following incubation at 37°C for 60 min, the filter is removed, cells on the upper surface wiped clear, and those on the lower surface fixed and stained. The extent of cellular migration is then readily quantified by image analysis, with the results expressed as a migration index (area of the lower surface of the filter occupied by lymphocytes in the presence of stimulant/area occupied by lymphocytes randomly migrating in the presence of medium alone). Although it has been claimed that lymphocytes do not adhere to polycarbonate filters that are not coated with collagen [2], we have shown that concentration-related agonist-induced responses are obtainable with uncoated filters if the above in vitro conditions are employed [1].

STIMULATION OF LYMPHOCYTE MIGRATION BY LIPID EXTRACTS

Ethyl acetate extracts of stratum corneum from psoriatic lesions were found to stimulate lymphocyte migration in a dilution-dependent manner, whereas similar extracts of normal heel stratum corneum, used as a control, were inactive. Recovery of the lipid activity from psoriatic stratum corneum was dependent on direct extraction with ethyl acetate, this material not being recovered by aqueous homogenization, as ethyl acetate extracts of aqueous supernatants were inactive in the assay. Purification by two straight phase HPLC systems shows that a major portion of the lipid activity coeluted with standard 12-hydroxyeicosatetraenoic acid (12-HETE) [3]. The activity was likely to be due mainly to 12(R)-HETE, as this has been shown to be the major 12-HETE isomer in psoriatic lesional stratum corneum and induces concentration-related responses in the lymphocyte migration assay. In contrast, standard 12(S)-HETE induced little or no response in the assay [1]. Furthermore, little or no activity above background was found in straight phase HPLC fractions eluting with leukotriene B₄. Although standard leukotriene B₄ produces concentration-related responses in the lymphocyte migration assay [1], its levels in the lesional samples tested and its potency in the assay were insufficient to stimulate lymphocyte migration.
This is in contrast to the results obtained with a neutrophil migration assay, which was of sufficient sensitivity to detect the leukotriene B4-like material in psoriatic lesional samples [4]. These findings, therefore, highlight the potential importance of 12(R)-HETE as a mediator of leukocyte infiltration in psoriasis. The evidence that the effects of 12(R)-HETE may be mediated via leukotriene B4 receptors on leukocytes [5] suggests that therapeutic trials of leukotriene B4 receptor antagonists in psoriasis may be worthwhile if they become available for clinical use.

STIMULATION OF LYMPHOCYTE MIGRATION BY AQUEOUS EXTRACTS

Aqueous extracts of psoriatic lesional stratum corneum stimulated lymphocyte migration in a dilution-dependent manner, and successive ultrafiltration through YM50 and YM10 membranes showed that the activity was predominantly >30 and <10 kD, with little activity in 10–30-kD fractions. Unexpectedly, aqueous extracts of normal heel stratum corneum produced a similar profile of activity, mainly in >30 and <10-kD but not in 10–30-kD fractions. Analysis of the same normal heel stratum corneum ultrafiltration fractions for interleukin 1 (IL-1) activity by an EL-4 NOB-1 assay [6] indicated the presence of substantial quantities of IL-1-like material in these 10–30-kD fractions [7]. The levels of IL-1 activity in these fractions appeared to be more than sufficient to stimulate lymphocyte migration, as determined by extrapolation from the responses to recombinant IL-1 alpha [8]. Thus the lack of lymphocyte chemotactant activity in the 10–30-kD fractions suggested the possible presence of an inhibitor of lymphocyte migration. The nature of this putative inhibitor and of the >30-kD lymphocyte chemotactant activity described above have not yet been resolved. However, the <10-kD activity has been subjected to detailed characterization, the initial work focusing on the activity in samples from normal skin because of their ready availability [9].

Apart from aqueous extracts of heel stratum corneum, dilution-related lymphocyte chemotactant activity was also found in chamber fluid samples from normal skin. These samples were obtained by a method involving the application of 2-cm-diameter acrylic cylinders to sellotape-striped normal skin and the addition of 1 ml sterile PBS for 30 min. Ultrafiltration showed that these chamber fluid samples also contained <10-kD activity. These findings, and the observation that material stimulating lymphocyte migration was also present in suction blister and friction blister fluids from normal skin, prompted the analysis of normal, heparinized human plasma, which was also found to induce reproducible, dilution-related responses in the lymphocyte migration assay. Serial ultrafiltration of plasma through YM100, YM10, and YM2 filters showed activity exclusively in YM2 filtrates, suggesting that the active material was nominally <1 kD. Reversed-phase HPLC, with acetonitrile gradient elution of chamber fluid and <10-kD ultrafiltrates of plasma and stratum corneum supernatants, consistently yielded a peak of lymphocyte chemotactant material eluting at about 25% acetonitrile. Simple ultrafiltration of plasma and chamber fluid samples followed by a single gradient elution reversed-phase HPLC step were sufficient to allow purification to homogeneity, as determined by several HPLC methods. UV absorbance spectrophotometry of highly purified plasma-derived material consistently showed a UV maximum at 274 nm, suggesting conjugated triene or diene-one structure. Further analysis is underway, but until a precise structure is available, the term "plasma-associated lymphocyte chemotactant" (PALC) has been proposed [9]. The presence of this lymphocyte migration-stimulating material in normal plasma suggests that it may play a role in physiologic lymphocyte trafficking by promoting margination and adherence to endothelial cells, and perhaps even migration of lymphocytes into the dermis if extravascular concentrations are high enough to provide a gradient. Its role as a mediator of pathologic lymphocyte infiltrates, for example, in psoriasis, remains to be investigated.

CONCLUSIONS

Aqueous extracts of stratum corneum samples from psoriatic lesions and normal heel induce similar activity in an in vivo lymphocyte migration assay. The nature of the larger molecular weight activity in the samples from either source, as well as its role in the induction of lymphocyte infiltrates in both normal and psoriatic skin, remain to be determined. Lower molecular weight material, for which the name PALS has been proposed, has been isolated from normal human plasma, similar or identical material also being identified in samples from normal skin. Its role in pathologic states has not yet been investigated, but it is suggested that it may function in physiologic lymphocyte trafficking. In contrast to the results of aqueous extraction, lipid extracts of psoriatic lesional stratum corneum consistently evoked greater stimulation of lymphocyte migration than samples from normal heel, and a major portion of the active material was found to be similar or identical to 12(R)-HETE. In addition to its neutrophil chemotactrant properties, this compound should be considered in the pathogenesis of the lymphocyte infiltrates of psoriasis.

REFERENCES

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