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EPIDERMAL CELL THYMOCYTE ACTIVITY FACTOR/INTERLEUKIN 1 (ETAF/IL)-LIKE ACTIVITY IN LYMPH DRAINED FROM NORMAL HUMAN SKIN

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ABSTRACT

Lymph derived from human skin contains lymphocytes which have a high rate of spontaneous blastic transformation in culture and are highly responsive to lectins. This phenomenon suggests that either a subpopulation of highly responsive lymphocytes is extravasated into skin, or skin tissue fluid and lymph contain humoral factors co-stimulating lymphocytes upon contact with tissue antigens. We sought to determine whether human prenodal lymph drained from normal leg skin possesses lymphokine activity. Significant augmentation of lectin induced thymocyte and autologous blood lymphocyte proliferation was produced by lymph. The augmenting activity was abrogated by incubation of lymph with anti-IL-1 antiserum. Supernatant from cultured lymph cells (lymphocytes, Langerhans cells) did not augment either thymocyte or autologous blood lymphocyte proliferation. No interleukin 2 activity was found in lymph. The data indicate that skin lymph possesses epidermal cell thymocyte activating factor/interleukin (ETAF/IL) 1-like activity which is not found in serum and that the main source of the putative lymphokine is epidermal and not migrating lymph mononuclear cells.

Abbreviations

ETAF: epidermal cell thymocyte activating factor
FCS: fetal calf serum
IL-1: interleukin 1
IL-2: interleukin 2
PBM: peripheral blood mononuclear cells
PHA: phytohemagglutinin

Lymph derived from human skin and subcutaneous tissue is a composite of capillary filtrate, substances released by skin cells and of immune cells which extravasate from blood capillaries. These cells migrate through the interstitial space and some enter the initial lymphatics. The high-molecular factors and cells accumulating in lymphatics are transported to the regional lymph node. Investigation of lymph, therefore, may provide insight into the immune events at the tissue level. We found previously (1) that concentration of immune proteins in the tissue fluid and lymph derived from skin is different from that of serum and that the differences depend on the molecular weight of individual proteins and the permeability of the capillary membrane. Moreover, the cellular composition of lymph differs from that of blood (2). In lymph there is a higher

representation of helper cells and lower of suppressor and B lymphocytes (3). Lymph contains migrating Langerhans cells (3) which are not observed in peripheral blood. Lymph lymphocytes also reveal high autotransformation rate in culture and increased responsiveness to mitogens (4). The differences in proportions of various cellular subsets and their functional capacities between lymph and blood indicate that not only does the capillary wall act as a filter for cell extravasation but also that the local extracapillary humoral environment regulates the process of extravasation and affects the function of cells which pass into the tissue space. In order to investigate whether humoral factors that exist within the tissue fluid affect the function of physiologically extravasated immune cells, we measured the level of activity of lymphokines in lymph draining normal skin and determined their influence on lymphocyte responsiveness.

MATERIALS AND METHODS

Subjects

Eight healthy male volunteers, aged 18-26 years, were studied.

Lymph collection

Lymph was collected from a superficial leg lymphatic. This vessel drained skin, subcutaneous tissue and perimuscular fascia of the foot and a part of the lower leg. The technique of lymphatic cannulation was described previously (5). Briefly, a lymph vessel running along the anterior aspect of the leg was dissected under strictly sterile conditions and polyethylene P60 Clay-Adams siliconized tapered cannula inserted into its lumen. The external tip of the cannula was placed in a sterile 10 ml plastic test-tube containing 0.5 ml of PBS with 20u of heparin without preservatives. The volunteers were allowed to walk normally.

Lymph cells

Lymph mononuclear cells were obtained from lymph samples collected over period of 6-12h by centrifugal sedimentation. Lymph serum was stored at -70°C.

Blood cells

Blood was drawn from the cubital vein. Cells were isolated by centrifuging on Lymphoprep (Nyegaard, Oslo) at 1600 r.p.m. for 35 min.

Mitogen assay of PBM cultured with lymph and serum

PBM were cultured in 200ul of RPMI 1640 containing 10% FCS at $2x10^5$ cells/well in round-bottom 96 well microtiter plates (Costar). PHA H15 (Wellcome) was added at final concentrations from 1.8 to 90.0μg/ml. Cultures were supplemented with 20% of lymph (protein concentration 18.0g/l) or serum (protein concentration adjusted to that of lymph). Cultures were pulsed at 48h with [^{3}H]TdR (specific-activity 2μCi/ml) and harvested at 72h.

Preparation of lymph cell and PBM supernatants

Lymph and blood mononuclear cells were incubated for 72h at 37°C in a humidified atmosphere at 10^6 cells/ml without or with 90μg/ml of PHA H15. After harvesting, supernatants were stored at -70°C.

Interleukin-1 assay in lymph and serum

C3H-HeJ thymocytes ($1.5x10^6$/well) were cultured in 96-well, round-bottom microculture plates for 72h in 100μl RPMI 1640 containing 5% FCS, antibiotics, 2.5x10-5M 2-mercaptoethanol, and 90μg/ml PHA. Dilutions of lymph or serum samples were added at 100ul volumes. Cultures in triplicate were harvested and incorporation of thymidine was measured in a Beckman liquid scintillation counter. Results were expressed

as units of activity. One unit was half-maximal counts per minute of test preparations compared with IL-1 standard assigned an arbitrary activity of 100u/ml.

Blocking studies

Anti-IL-1 polyclonal antibody (Cistron, N.J.) was incubated at 20% concentration (v/v) with dilutions of lymph and serum samples for 12h at 4°C. Samples before and after such treatment were assayed for IL 1 activity.

In order to study the heat sensitivity of IL-1, the lymph and serum samples and lymph and blood cell culture supernatants were kept for 2h in a 56°C water bath.

Interleukin-2 assay

The IL-2 activity was determined using IL-2 dependent T-cells (CTLL 2). The activity of IL-2 was measured against a standard of recombinant IL-2 (Genzyme, Haverhill).

Immunohistological staining of lymph cells for detection of IL-1

Lymph cell smears were treated with anti-human IL-1 mouse monoclonal antibody (Cistron) and subsequently with rabbit-anti-mouse and swine-anti-rabbit alkaline phosphatase conjugated antibodies (DAKO, Copenhagen).

Statistical evaluation

Values were expressed as means ± standard deviation. For evaluation of the statistical significance of differences between the effects of lymph and serum Student-t-test for pairs was used.

RESULTS

Responsiveness of PBM to PHA in culture with lymph and serum

The responsiveness of PBM in cultures supplemented with lymph was higher at all concentration of PHA than in culture with serum *(Fig. 1)*. It ranged from 50% at low to 7% at high

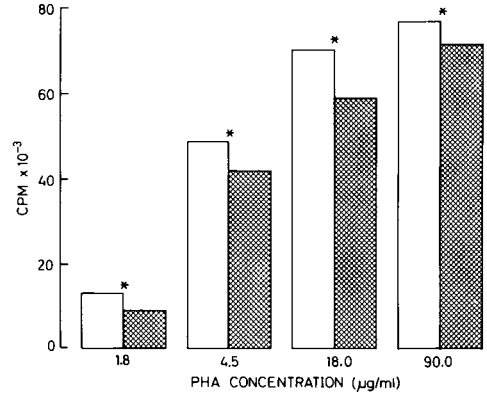

*Fig. 1. The effect of normal human prenodal lymph on PBM in culture using 20% of autologous lymph (OPEN BAR) or serum (GRID) and increasing concentrations of PHA. Mean values are from 8 subjects, * p<0.05. Note that lymph at each concentration augments PBM proliferation more than serum.*

PHA concentrations. The responsiveness of PBM in cultures with DFCS was similar to that with autologous serum.

IL-1-like activity in lymph and serum

Lymph revealed thymocyte co-stimulating activity in the presence of PHA, which was not observed in serum *(Fig. 2)*. The mean peak IL 1-like activity at

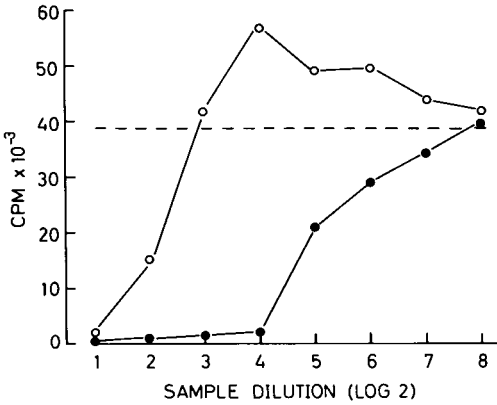

Fig. 2. The IL-1 assay with C3H/HeJ thymocytes in lymph (OPEN CIRCLE) and serum (CLOSED CIRCLE). Interrupted horizontal line represents the mean level of background incorporation in the absence of either lymph or serum. At low dilutions of test lymph and serum samples an inhibitory effect is seen. $4x10^3$ c.p.m. correspond to 1 IL-1u/ml. Mean values from 8 subjects.

dilution of 1:16 was 4.4u/ml and at dilution of 1:256 1.5u/ml. At low dilutions, both lymph and serum exhibited an inhibitory effect on thymocyte proliferation, most likely due to the presence of IL-1 inhibitor.

Blocking effect of anti-IL-1 antiserum

The polyclonal anti-IL-1 antiserum totally abrogated the IL-1-like activity in lymph *(Fig. 3)*. Control rabbit serum

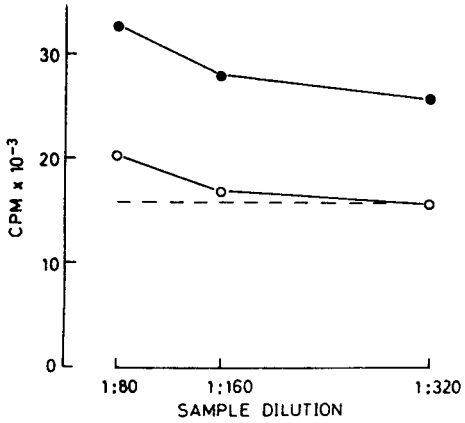

Fig. 3. The blocking effect of anti-IL-1 antiserum on thymocyte proliferating properties of lymph. Antiserum treated (OPEN CIRCLE) and control (CLOSED CIRCLE) samples. Interrupted horizontal line represents the mean level of background incorporation in the absence of lymph. Mean values from 4 subjects.

did not exert such inhibitory effect. Heating lymph samples at 56°C for 2h decreased the IL-1-like activity at sample dilution of 1:80 by 40% and at 1:160 by 19%.

Stimulatory effects of lymph and blood cell culture supernatants on PBM responsiveness and thymocyte proliferation

Lymph cell culture supernantants did not stimulate PBM cultured with 1.8μg/ml of PHA, whereas supernatants from PBM cultures exhibited stimulatory properties (stimulation index 1.6-3.1).

In the thymocyte proliferation assay no IL-1 activity could be detected in lymph cell supernatants *(Fig. 4)*. The

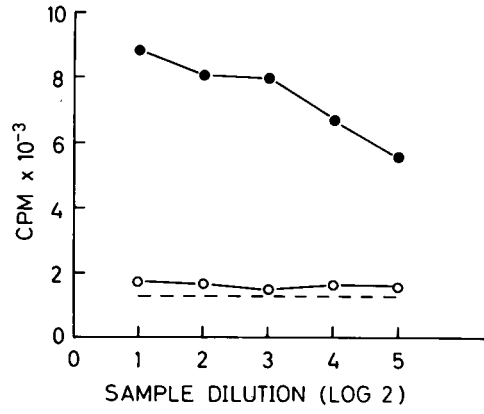

Fig. 4. The IL-1 assay in PBM (CLOSED CIRCLE) and lymph (OPEN CIRCLE) cell culture supernatants. Interrupted horizontal line represents the mean level of background incorporation in the absence of supernatants. Mean values from 6 subjects. Note the lack of IL-1 activity in lymph cell culture supernatants.

PBM cell culture supernatants stimulated thymocyte proliferation with a mean index of 7.2.

IL-2 assay in lymph and serum

No IL-2 activity was detected either in lymph or serum.

Histochemical test on detection of IL-1 in lymph cells

No membrane-associated or cytoplasmic IL-1 was detected in lymph lymphocytes or Langerhans cells.

DISCUSSION

This study yielded the following information: a) prenodal lymph drained from the foot skin of normal man had an enhancing effect on the responsiveness of autologous PBM to PHA, b) lymph stimulated proliferation of C3H/HeJ mouse thymocytes, c) the stimulated proliferation of mouse thymocytes was abrogated by incubation of lymph with anti-IL-1 antiserum, d) supernatants from cultured lymph cells (lymphocytes, Langerhans cells) did not stimulate either autologous lymphocytes or mouse thymo-

cytes, e) no IL-2 activity was found in lymph.

The putative cytokine present in lymph and responsible for the enhanced responsiveness of PBM to PHA and for the thymocyte proliferation seems to be ETAF/IL-1 (6,7). There are evident similarities between the biological effects of ETAF/IL-1 and those observed by us in cultures supplemented with lymph. ETAF/IL-1 promotes proliferation of T cells (8,9) which was also observed in our experiments. Using antibodies against IL-1, we were able to inhibit the lymph stimulatory activity, which is consistent with the findings of Sauder et al. (10) who blocked ETAF activity with antiserum against monocyte derived leukocytic pyrogen. ETAF/IL-1 is thermostable at temperatures below 60°C but sensitive to higher temperatures (6). We found that the cytokine present in lymph was sensitive to prolonged exposures to 56°C.

Efforts to isolate the pure putative cytokine from lymph have so far proved unsuccessful due to the difficulties in its separation from the nondialysable inhibitor. Analysis of the activity curves indicates that the cytokine was detectable only when lymph samples were assayed at high dilution. This was due to the presence of inhibiting substances most active in whole nondiluted serum. Similar findings were described by Wood et al. (11). Miossec (12) observed that IL-1 activity was low when endothelial cells were cultured in the presence of serum. Saunder et al. (7) observed the presence of a small molecular weight inhibitor of DNA synthesis in epidermal cell culture supernatants.

There was no IL-2 activity in lymph which rules out the possibility that this cytokine is responsible for the co-stimulatory effects on lymphocytes and thymocytes.

The possible sources of the ETAF/IL-1-like cytokine in lymph drained from skin may be: a) epidermal keratinocytes (6), b) epidermal resident (1) and migrating Langerhans cells, c) skin endothelial cells (12), d) plasma capillary filtrate.

The supernatants of lymph cells cultured in the presence or absence of PHA did not contain ETAF/IL-1-like activity. The percentage of Langerhans cells in prenodal lymph is 3-8% and of monocytes (OKM 1+) 1-2%. Thus, the density of these cells seems to be too low to contribute significantly to the cytokine pool of lymph. Also no IL-1 is found on the membrane or in the cytoplasm of Langerhans cells, although membrane-associated IL-1-like activity has been detected by others on rat dendritic cells (13) and human monocytes (14). This finding makes it highly unlikely that lymph cells are contributors of ETAF/IL-1 activity.

The lymphatic endothelial cells may be a source of IL-1-like cytokine found in lymph; however, no direct studies were done. Nonetheless, a relatively low number of these cells in the tissue studied and lack of any stimulatory factors for production and release of IL-1 in the normal skin makes this possibility remote.

Since no ETAF/IL-1-like activity was detected in serum, so too its capillary filtrate, which after passage through the interstitium becomes lymph, is unlikely to possess this activity.

The most probable origin of ETAF/IL-1-like activity in lymph seems therefore to be epidermis. However, the question arises as to whether this factor may be a constitutive product of epidermis. The classical IL-1 producers do not contain this factor as such but produce it when cultured and stimulated. A similar finding was observed when keratinocytes and Langerhans cells were studied for IL-1 activity (6,10). In our studies, we found ETAF/IL 1-like activity in lymph drained from normal non-stimulated skin. Recently, Hauser et al. (15) have detected IL-1 and its possible precursor in normal non-stimulated epidermis.

In conclusion, normal human prenodal lymph derived from skin possesses an ETAF/IL-1-like activity which is not found in serum, and the chief source of the putative lymphokine is epidermal and

not migrating mononuclear cells. The biological implications of this ETAF-IL-1-like factor is unclear, but this cytokine may modulate immune responses not only locally in the skin but also in regional lymph nodes.

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