Lymphology 15 (1982) 168–173

Cells in Lymph Draining Normal Human Skin-monoclonal Antibody Analysis

W.L. Olszewski, I. Grzelak, Arnfinn Engeset

Laboratory for Hematology & Lymphology, Norwegian Radium Hospital and Norsk Hydros Institute for Cancer Research, Oslo and Dept. of Surg. Res. & Transplantology, Medical Research Center, Polish Academy of Sciences, 02004 Warsaw

Summary

The immune cells which migrate into the human skin from the blood and subsequently leave it via lymph vessels play an important role in immune processes. We made use of the monoclonal antibodies, characterizing cell populations which migrate into the normal skin and which having traversed the tissue, could be recovered from the afferent lymph vessels. The percentage of $OKM1^+$ cells (monocytes/macrophages, null cells) in lymph was low (8.9 ± 1.6%) when compared to that of blood (16.5 ± 4.6%) ($p < 0.05$). The OKM1 antibody labeled only 40% of the large macrophage-like lymph cells. The percentage of $OKT3^+$ (T cells) in lymph was higher (75.4 ± 4.0%) than in blood (54.0 ± 4.5%) ($p < 0.05$) as was that of the $OKT4^+$ (inducer/helper) subset (41.5 ± 9.5 and 33.3 ± 4.8%, $p < 0.05$), while cells of the $OKT8^+$ (suppressor/cytotoxic) subset were found to be less numerous in lymph than in blood. (18.4±6.2% and 20.3±4.9%, $p<0.05$). The OKI $a1^+$ cell population consisted of large veiled macrophage-like cells and only very few small cells. Around 60% of the large mononuclear cells present in lymph reacted with OKT6 antibody specific for cortical thymocytes. The finding of high proportions of T cells, cells bearing Ia-like antigens, and a high inducer/suppressor ratio in normal prenodal lymph reflects the intensity of "physiological" immune processes in the normal skin.

The immuno-competent cells which migrate into the human skin from the blood, and subsequently leave it via lymph vessels, play an important role in immune processes. These cells eliminate bacterial antigens and virus-infected or neoplastically transformed cells (1), prevent the induction of unresponsiveness to antigen placed on cutaneous surfaces (2), and when transferred with skin grafts as "passenger lymphocytes" to an allogeneic recipient evoke a vigorous and difficult to control reaction of the host (3, 4, 5). There appears to be some selection of migrating cells at the level of the capillary bed, by which certain cell types are selected to enter the skin (6, 7, 8). We made use of the monoclonal antibodies (9–13), to characterized the cell populations which after migrating into the normal human skin and traversing the tissue, could be recovered from the afferent lymph vessels. Significant differences were apparent between the types and proportions of cell populations in lymph and blood.

Materials and Methods

Subjects

Twelve healthy male volunteers, aged 18–26 years, were studied.

Lymph cell collection

Lymph was collected from a leg superficial vessel. This vessel drained the skin, subcutaneous tissues and perimuscular fascia of the foot and a part of the lower leg. The technique of lymphatic cannulation was described previously (14, 15). Briefly, a lymph vessel running along the anterior aspect of the leg was dissected under strictly sterile conditions and polyethylene P60 Clay-Adams siliconized

This work was supported by grants from the Norwegian Cancer Society and the Polish Academy of Sciences. We thank the Davis & Geck, American Cyanamid Co. for aid.

0024–7766/82 040168–06 $ 02.00

tapered cannula inserted into its lumen. The external tip of the cannula was placed into a sterile 10 ml palstic test-tube containing 1 ml of PBS with 20 units of heparin without preservatives. The volunteers were allowed to walk normally.

Lymph mononuclears were obtained from lymph samples collected over periods of 6–12 h by centrifugal sedimentation. Since the lymph cell population contained no granulocytes and only very few erythrocytes, no density gradient separation was necessary.

Blood mononuclear cell isolation. Blood samples were taken from the cubital vein. Mononuclear cells were isolated by centrifugation on Lymphoprep (Nyegaard, Oslo) at 1600 rpm for 35 min. Cells from the interface were collected, washed three times in RPMI 1640, and adjusted to appropriate concentrations.

Monoclonal antibodies

They were obtained from Ortho Pharmaceuticals (Raritan, New Jersey). One type, OKM1 reacts with 78% of adherent mononuclear cells (9) and 18% of non-adherent cells (null-cells), another, OKT3 with 100% of peripheral T lymphocytes (10), OKT4 with 65% of T lymphocytes (11), the subpopulation expressing the inducer/helper phenotype, and OKT8 with 35% to T lymphocytes, the subclass of suppressor-cytotoxic cells (12). The OKIa1 antibody reacts with 90% of B lymphocytes and monocytes and 20% of null cells, as well as, with activated T lymphocytes (13) and the OKT6 antibody with 70% of human thymocytes.

Identification of cell subpopulations with monoclonal antibodies. Lymphocytes were suspended in RPMI 1640 medium (Gibco), supplemented with 5% heat-inactivated foetal calf serum (DFCS) and 25 mM HEPES, at a concentration of 5 x 10^6 cells/ml and maintained at 0–4 °C. Aliquots of two hundred microliter were incubated with 5 µl of reconstituted monoclonal antibody preparations. After a 30 min incubation in an ice-water bath cells were washed RPMI 1640 medium with HEPES supplemented with DFCS, resuspended in 100 µl of the same medium and mixed with 100 µl of a 1:20 dilution of fluorecein conjugated goat anti-mouse gamma globulin $(Fab)_2$ fraction (N.L. Cappel, Cochranville, PA). After a further incubation for 30 min in ice-water bath, cells were washed as before and then resuspended in 1–2 drops of mounting medium (PBS, pH 7.2, containing 30% v/v of glycerol). The percentage of fluorecent positive cells in each sample was determined by examining 200 randomly chosen cells with the aid of a fluorescent microscope. The morphological appearence of fluorescent cells was evaluated.

Statistical methods

Values were expressed as means ± standard deviation. For evaluation of statistical significance of differences in results where a comparison between values for lymph and blood was made, the Wilcoxon matched pairs signed ranks test was used.

Results

$OKM1^+$ cells

The mean percentage of all lymph cells labeled with OKM1 antibody was 8.9 ± 1.6, of blood mononuclears 16.5 ± 4.6 ($p < 0.05$) (Fig. 1). There was 7.38 ± 2.4 percent of large, mononuclear, macrophage-like cells and 1 ± 0.76 percent of small cells with scanty cytoplasm. However, of all macrophage-like lymph cells only about 40% reacted with the OKM1 antibody. In the blood the majority of $OKM1^+$ cells were medium-size mononuclears with a kidney-shape nucleus, and no large cells like those in lymph could be detected.

$OKT3^+$ cells

The mean percentage of lymph cells labeled with OKT3 antibody was 75.4 ± 4.0, of blood cells 54.9 ± 4.5 ($p < 0.05$) (Fig. 2). In both, lymph and blood the $OKT3^+$ cells were small, round-shape nucleus cells with scanty cytoplasm.

$OKT4^+$ and $OKT8^+$ cells

Lymph contained 41.5 ± 9.5 percent of helper/

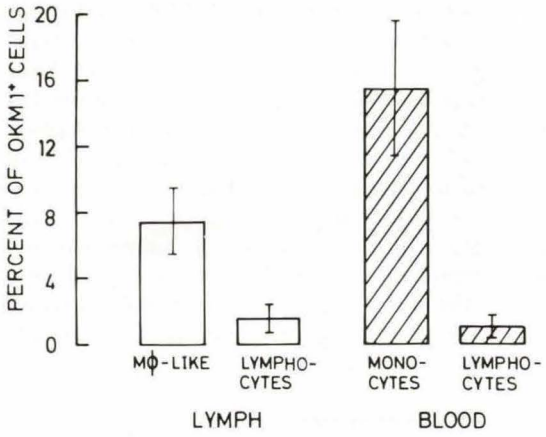

Fig. 1 Mononuclear cells in human prenodal lymph and blood reacting with OKM1 monoclonal antibody. Lymph cells – open bars, blood cells – shaded bars. Values are means ± SD of 20 tests in 10 normal men.

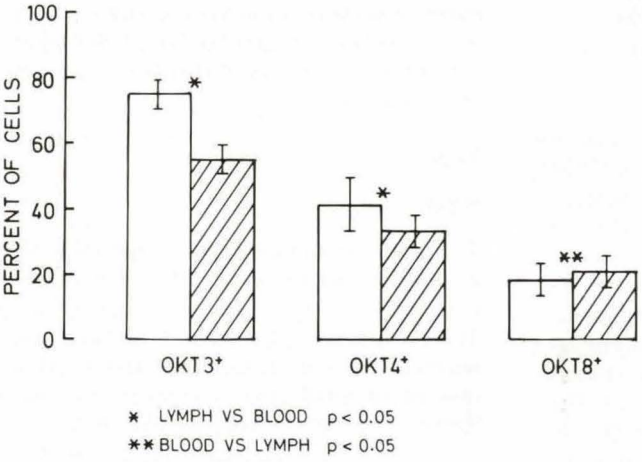

Fig. 2 Mononuclear cells in human prenodal lymph and blood reacting with OKT3, OKT4 and OKT8 monoclonal antibodies. For details see Fig. 1.

inducer cells ($OKT4^+$), whereas blood contained 33.3 ± 4.8% ($p < 0.05$) (Fig. 2).

Suppressor/cytotoxic ($OKT8^+$) cells were represented in lymph in 18.4 ± 6.2%, in blood in 20.9 ± 4.9% ($p < 0.05$) (Fig. 2). Both antibodies, OKT4 and OKT8 labeled only small with round-shape nucleus cells.

In so far as the $OKT4^+$ and $OKT8^+$ subsets mainly comprise the T cell population, the combined percentage of these cells should equal the figure for $OKT3^+$ cells. This was found to be true in our studies in the case of T lymphocytes derived from blood whereas in lymph , 19.6% ± 15.2 ($p < 0.0006$) $OKT3^+$ cells did not react with either of the OKT4 and OKT8 antibodies.

The mean ratio of the $OKT4^+/OKT8^+$ subsets was 2.26 in lymph and 1.59 in blood ($p < 0.05$).

$OKIa1^+$ cells

The OKIa1 antibody labeled two types of cells in lymph: 3.0 ± 5.2% of small mononuclear cells and 9.2 ± 5.6% large, veiled, macrophage-like cells (Fig. 3). Of all the large cells about 24% did not react with the OKIa1 antibody. In blood, small mononuclear cells comprised 7.3 ± 5.0%, and monocytelike ones 4.5 ± 6.0%.

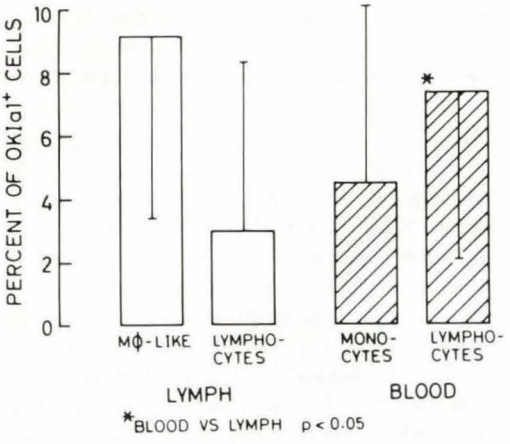

Fig. 3 Mononuclear cells in human prenodal lymph and blood reacting with OKIa1 monoclonal antibody. For details see Fig. 1

The OKIa1$^+$ blood mononuclears belonged most certainly to the monocyte and B cell subsets.

OKT6$^+$ cells

Lymph contained 5.9 ± 3.5 % of OKT6$^+$ cells. The OKT6 antibody labeled only large, macrophagelike cells and only about 60 % of that population. No OKT6$^+$ cells were found in blood.

Discussion

This study has yielded the following information: a) the percentage of OKM1$^+$ cells in lymph was low when compared to that of blood, b) the percentage of OKT3$^+$ cells in lymph was higher than in blood as was that of the OKT4$^+$ subset, while cells of the OKT8$^+$ subset were found to be more numerous in blood, c) the OKIa1$^+$ cell population in lymph consisted of the majority of large veiled mononuclear cells and only few small cells. The former were not detected in blood, d) about 60 percent of large mononuclear cells present in lymph reacted with the OKT6 antibody, i.e. that specific for cortical thymocytes. No OKT6$^+$ cells were found in blood. Presumably, all the above characterized cells play a role in the local immune surveillence in the skin.

The method used thus far to characterize lymph mononuclear cells have been based on determining rosette formation ability and the detection of surface immunoglobulins. Such methods could not be applied to give a precise insight into the populations migrating through the tissues. By using the monoclonal antibodies, we were able to discriminate between the morphologically similar lymph monocytes and lymphocytes, to estimate the percentage of T cells and their helper and suppressor subsets and of cells bearing Ia-like antigens, and to characterize the surface antigens of lymph macrophage-like cells. Moreover, we could compare both qualitatively and quantitatively the lymph and peripheral blood populations, thereby obtaining information concerning the type of cells traversing the normal skin and subcutaneous tissue.

However, when comparing differences in observations made on cells derived from respectively lymph and blood, the question remains to be answered as to whether the lymph cell population which was not isolated on a density gradient could be compared with the blood cell population separated on Lymphoprep. We have found that isolation of blood cells on Lymphoprep brings about a quantitative loss of mononuclears without significantly affecting the proportions of the different subsets.

The percentage of OKM1 labeled cells in lymph was found significantly lower than in blood. The OKM1 antibody labeled about 40 percent of the large macrophage-like cells, some of which are supposed to be the migrating skin Langerhans cells, typical of prenodal lymph. However, because some 60 % of the macrophage-like lymph cells did not react, this antibody appears to indicate the existence of two subsets of cells with an almost identical morphological appearence.

The low level of OKM1$^+$ cells in lymph should be indicative of low natural killer (NK) cell activity in as much as NK cells belong to the null cell population which reacts specifically with OKM1 antibody. This observation agrees with previous work in which we described the

characteristically low NK cells activity in lymph (8).

The overall low number of $OKM1^+$ cells in lymph could not be an artefact caused by their loss due to adherence to the cannula employed in collecting lymph, since microscopic examination of the inner walls of the cannula revealed that very few cells did in fact attach. The low percentage of small $OKM1^+$ cells in lymph could rather be caused by a limited extravasation rate of these cells from blood capillaries. The large macrophage-like $OKM1^+$ cells could partly derived from the blood monocytes, partly represent the Langerhans cells normally residing in the skin.

The percentage of the $OKT3^+$ cells in lymph evidently exceeded that of blood. This observation further confirms the validity of our previous results (8), in this case, where E-rosette formation technique was used to quantify the T cells. That the lymph is relatively more enriched in T cells than blood might be the consequence of the generally low level of monocytes and B cells in lymph (6, 7, 8). The question as to whether the difference in the proportions of T cell and monocyte populations in lymph and blood relates to an increased predilection for T cells to extravasate in comparison to monocytes and B cells remain at present unknown.

The $OKT4^+$ cell population was more prevalent in lymph than in blood. The lower figure for blood is relative rather than absolute since these cells will be more diluted in blood than lymph by non-T cells. The $OKT8^+$ cells were less predominant in lymph than in blood. A subset of $OKT3^+/OKT4^-$ and $OKT3^+/OKT8^-$ cell was detected in lymph which could not be identified in blood. This subset remains to be characterized and further attention will be given to it in future studies.

The ratio of the $OKT4^+/OKT8^+$ subsets is often used as a means of demonstrating the predominance of helper subsets over suppressor ones, or for indicating a selective depletion of the suppressor population in clinical conditions. In our studies the mean $OKT4^+/OKT8^+$ ratio was 2.26 in lymph and 1.59 in blood. The high helper/suppressor ratio in lymph was accountable to the high level of inducer/helper cells rather than to the relatively low distribution of suppressor/cytotoxic cells.

Lymphocytes migrating through the interstitial space may come into direct contact with the antigenic determinants, whether of bacteria which have traversed the epithelium or virus-infected autologous cells, or indirect contact through the tissue macrophages processing these antigens. Information which should be transferred to the regional lymph node may thereby be acquired. This function is that of the inducer/helper population and thus it is to be expected that this subset should be first to respond to stimulation, and that the suppressor subset be activated only at a later stage so as to balance the functional equilibrium existing between the cooperating T cells. This notion is supported by data documenting a high spontaneous blastic transformation rate of the $OKT4^+$ population of prenodal lymph T cells when compared to that of the $OKT8^+$ population (16).

The OKIa1 antibody labeled in lymph the majority of large, macrophage-like, veiled cells and about 3.0 percent of small cells. The presence of Ia-like antigens on the surface of Langerhans cells in lymph (17, 18) may be important in the binding of sensitizing agents to these antigens during the induction of a local T lymphocyte cytotoxic reaction (19) or transportation of antigens from the skin to the lymph nodes. We have seen lymphocytes, in lymph, being attached to large mononuclear cells, an observation which strengthens the concept of there existing a degree of cooperation between these two cell types in vivo. The small mononuclear $OKIa1^+$ cells in lymph most likely belonged to the monocyte and B-cell subsets, and, some were probably stimulated T-cells. Stimulated T lymphocytes acquire Ia-like antigens (20) and we have found that lymphocytes from prenodal lymph reveal a high degree of spontaneous activation, even after 24 h culture (16).

The majority of large veiled cells in lymph reacted with OKT6 antibody. This antibody reacts with 70% of thymocytes, but not with

circulating T lymphocytes or monocytes. This observation has important implications: firstly, it raises the question of a possible immunological relationship between thymus and skin, and in fact, Langerhans cells have been shown to be present in the human thymus by studies utilizing electron-microscopy (21); secondly, it further strengthens the view that Langerhans cells are of hematogeneous origin.

In conclusion, this study has shown that lymph derived from normal human skin provides us with populations of cells whose surface markers and functional state reflect the "normal" tissue immune status. Certainly, not all migrating cells which extravasated from blood capillaries during the collection period would necessarily be recovered in the lymph, since some may have remained in the interstitial space, or perished there, while others would have entered the epithelial layer to become the "interepithelial lymphocytes". Nevertheless, the populations found in lymph displayed enough antigenic differences when compared to blood mononuclear cells as to demonstrate the character and importance of the "tissue filter" for the migrating lymphoid cells.

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W.L. Olszewski, Lab. for Hematology & Lymphology, Norwegian Radium Hospital, Oslo 3, Norway