LYMPHOCYTE MIGRATION INTO SYNGENEIC IMPLANTED LYMPH NODES

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UDC 616.428-089.843-07:
616.428-008.953.2-07

KEY WORDS: syngeneic implantation; homing; migration; reticular stroma.

Several problems concerned with the histogenetic relations between the stroma of hematopoietic and lymphoid organs and cells colonizing them have been studied on models of implanted organs and tissues [1-3, 6, 8, 9, 13].

In the investigation described below a method of subcutaneous implantation of peripheral lymph nodes (LN) was used to study the character of migration of lymphocytes into a lymphoid organ regenerating after transplantation. The particular problem chosen for study was how the property of circulating lymphocytes, to return and colonize particular lymphoid organs (homing), is realized by the microenvironment of regenerated lymphatic tissue, and what structures are responsible for controlling migration and outflow of lymphocytes from LN. This organ-specific migration was demonstrated by determining the localization of ^{51}Cr-labeled lymphocytes injected intravenously.

To judge the functional activity of lymphocytes of an implanted LN the proliferative response of lymphocytes of the implanted organ in the blast-transformation reaction *in vitro* and their ability to induce a local graft versus host reaction (GVHR) were determined.

EXPERIMENTAL METHOD

Male CBA mice aged 2.5-5 months, obtained from the "Stolbovaya" Nursery, Academy of Medical Sciences of the USSR, were used as both donors and recipients. Under aseptic conditions peripheral LN (popliteal, inguinal, submandibular, cervical, axillary, cubital) were removed from the mice used as donors of the implanted material, transferred into medium 199 with 10% embryonic calf serum on ice, and transplanted subcutaneously into the chest and abdominal wall of recipient mice, at the rate of two LN into each animal.

The lymphocyte suspension for labeling with ^{51}Cr was obtained from peripheral LN in different situations (see above) from syngeneic (CBA) mice. The resulting lymphocyte suspension was labeled [13] with a solution of sodium chromate-^{51}Cr in a concentration of 20-40 μCi/100·10^6 cells in 1 ml for 40 min at 37°C. The cells were washed three times and injected intravenously into recipients with implanted LN, at the rate of 10^7 cells in a volume of 0.5 ml into each recipient. The following organs were removed 4 and 20 h after injection of the labeled lymphocytes into the recipients for radiometry: the implanted LN, the recipients' own (inguinal) LN, the spleen, liver, blood, skin (dorsal), and muscle tissue.

The distribution of labeled lymphocytes in each organ was determined by counting radioactivity, in counts per milliliter, on a Gamma counter (Packard, USA), and the results were expressed as percentages of radioactivity of the total dose of injected labeled cells (usually 30·10^3 to 40·10^3 cpm).

Some syngeneic implants were subjected to histological analysis at different times after transplantation (after 1, 2, 5, 7, 14, 21, 30, and 80 days, and 8 months). In addition, a group of implanted LN, transplanted into allogeneic (CBA → C57BL) recipients, was studied. After fixation with Carnoy's mixture and formalin, sections were cut to a thickness of 5 μ, and stained with methyl green — pyronine, and impregnated with silver by Gomori's method to detect argyrophilic fibers.

Laboratory of Natural Immunity, N. F. Gamaleya Research Institute of Epidemiology and Microbiology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 460-463, October, 1985. Original article submitted December 29, 1985.

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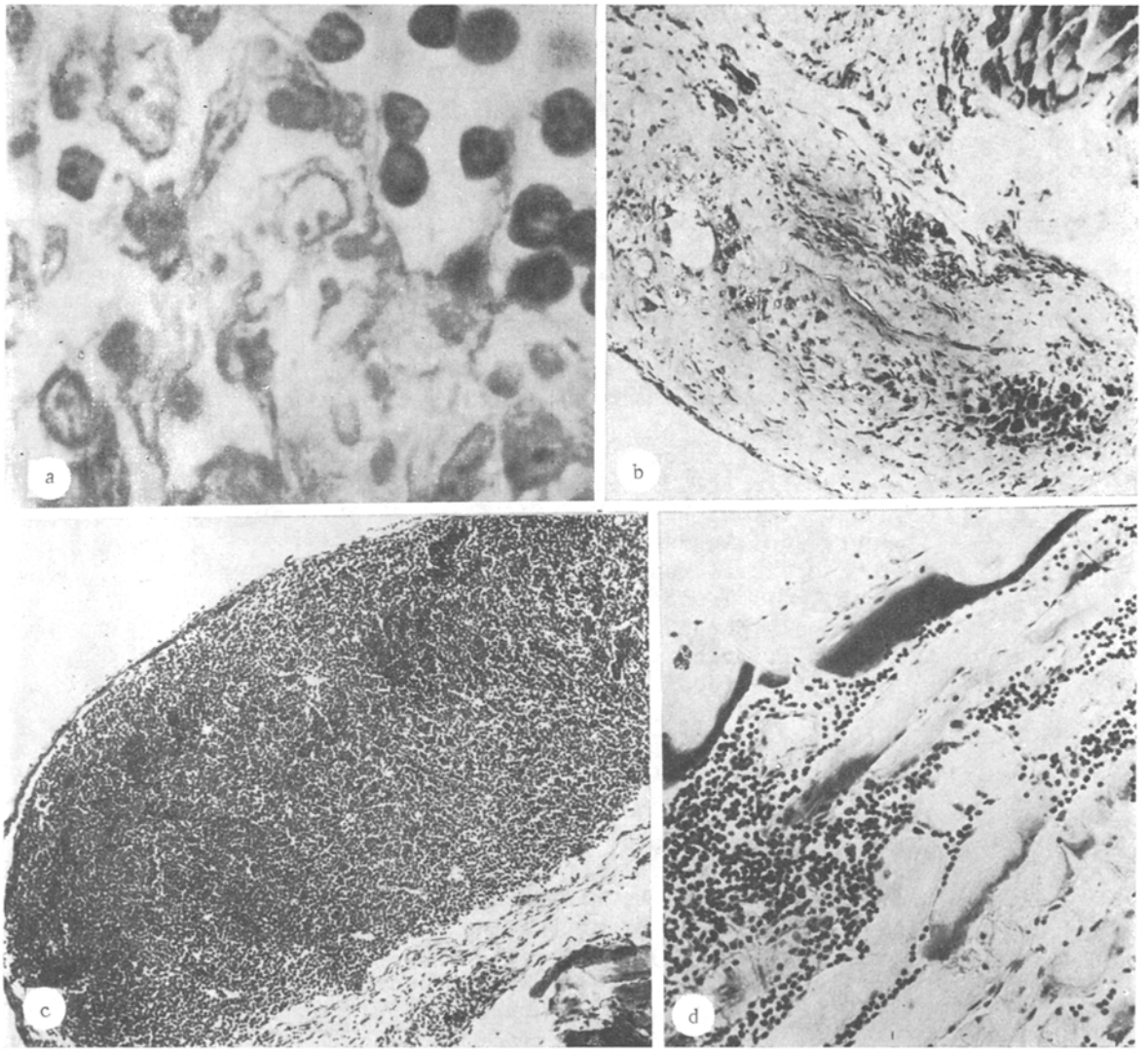

Fig. 1. Morphology of implanted LN. a) Implanted syngeneic LN 2 days after transplantation: groups of lymphocytes at periphery among reticular stromal cells. Methyl green–pyronine; 2000 ×; b) implanted allogeneic LN 14 days after transplantation. Stained with hematoxylin and eosin; 106 ×; c) implanted syngeneic LN 80 days after transplantation: dense lymphatic tissue fills the whole implant. Stained with hematoxylin and eosin; 106 ×; d) tissue of implant bed 60 days after transplantation: outflow of lymphocytes into muscle tissue. Methyl green–pyronine; 208 ×.

In the next group of experiments the proliferative response of lymphocytes in 30-day-old implants was studied in the blast transformation reaction *in vitro* with the following mitogens: concanavalin A (Con A), from Pharmacia (Sweden), and lipopolysaccharide (LPS), from Sigma (USA).

A suspension of lymphocytes obtained from the implants and from the recipients' own (peripheral) LN were cultured by a micromethod [10] in plastic microplates. The culture medium was medium RPMI-1640 with the addition of 5% horse serum, 0.0028 mM 2-mercaptoethanol, 10 mM HEPES, 2 mM L-glutamine, and antibiotics. The proliferative activity of the lymphocytes was estimated as incorporation of ^{3}H-thymidine into DNA during incubation of the cell suspension for 3 days. ^{3}H-Thymidine was added in a dose of 0.5 μCi per well of the microplate 16 h before the end of culture (specific radioactivity 1 Ci/mmole).

Experiments to study lymphocyte migration and the proliferative response were repeated at least three times.

To carry out the local GVHR, a cell suspension was obtained from a pool (8 to 10) of 30-day syngeneic implanted LN from CBA mice, the cells were washed with medium 199, and injected into the hind footpad of (CBA × C57BL)F_1 hybrids in a dose of $8 \cdot 10^6$–$10 \cdot 10^6$ lymphocytes

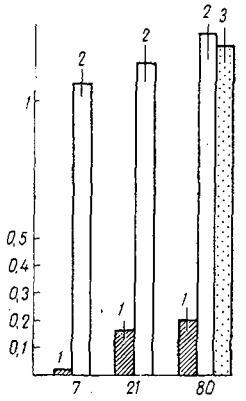

Fig. 2

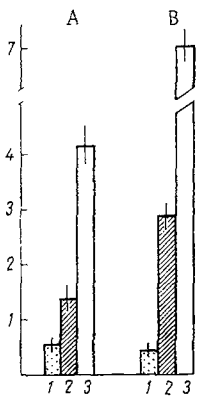

Fig. 3

Fig. 2. Migration of ^{51}Cr-labeled lymphocytes into implanted LN in syngeneic recipients and into their own LN. Abscissa, time after transplantation (in days); ordinate, radioactivity (in % of total radioactivity of injected lymphocytes). 1) Implanted LN; 2) recipient's own (inguinal) LN; 3) inguinal LN of intact mouse. When calculating percentage of radioactivity in an implanted LN or recipient's own LN, weighing from 1.5 to 2.5 mg, the mean value was calculated for 16-20 LN in each group respectively. Radioactivity in the skin and muscle tissue was 0.001 or 0.01 and 0.0007% per 100 mg tissue.

Fig. 3. Proliferative response of lymphocytes of implanted LN and of recipients' own LN to mitogens. Ordinate, radioactivity (in cpm × 10^3). Abscissa: 1) control (culture without mitogen); 2) LPS; 3) Con A. A) Lymphocytes of 30-day implants; B) recipient's lymphocytes.

in a volume of 0.05 ml. At the same time, 0.05 ml of medium 199 was injected into the contralateral (control) limb. The results were read on the 7th day after injection of the cells, as the degree of change in weight of the popliteal LN, obtained by dividing the experimental weight by the control weight. These results were compared with those of the GVHR, obtained after injection of the same number of cells of the recipients' own peripheral LN into the hybrids.

EXPERIMENTAL RESULTS

Morphological analysis revealed that after massive death of cells, mainly lymphocytes, in the syngeneic implanted LN, groups or concentrations of lymphocytes remained viable during the first 24 h after transplantation at the periphery of the implant, against the background of areas of depopulated reticular stroma (Fig. 1a). The central part of the implants was filled with necrotic masses. Later, as the blood vessels grew in and necrotic masses occupying the central part of the implants underwent resorption, 70% of the implants regenerated. Allogeneic implants were rejected by the 12th-16th day in 100% of cases, and replaced by fibrous connective tissue (Fig. 1b). The syngeneic implants 3 weeks after transplantation were filled with dense lymphatic tissue, resembling the cortical zone of intact LN, but without follicles (Fig. 1c) and with sinuses (including marginal) filled with lymphocytes. The system of sinuses had no definite orientation such as is characteristic of LN. The wide efferent duct also was absent. The medullary cords of the medulla either were absent or had generated partially and in small numbers. Concentrations of plasma cells were observed in the perivascular connective tissue along the course of larger blood vessels of arterial type. Reticulin fibers, leaving the capsule, formed a fairly homogeneous network of medium-sized loops, with an increase in number of collagen fibers.

These experiments showed that migration of lymphocytes into implanted LN is drastically reduced. For instance, the level of migration of lymphocytes into the implanted LN on the

30th day after transplantation was more than 10 times lower than the level of migration into the recipients' own LN and into LN of intact animals (Fig. 2).

A study of the proliferative response of lymphocytes of 30-day-old implants revealed that their mitogenic activity was 33-50% less than that of lymphocytes of the recipients' own peripheral LN and LN of intact animals (Fig. 3). The response of lymphocytes of the implant to Con A was 50% lower, and that to LPS was 33% lower than the proliferative response of lymphotyces of the recipients' own LN. Activity of lymphocytes of 20-30-day-old implants in the local GVHR did not differ significantly from activity of lymphocytes of the recipients' own LN. The ratio of the weight of the experimental LN to the weight of the control LN was 5.225 ± 0.436 and 5.665 ± 0.590 respectively.

It was thus shown that syngeneic implants, transplanted subcutaneously, regenerate, but the structure of intact LN is not fully restored.

Regeneration of lymphatic tissue in the implant took place on the basis of residual areas of stroma and repopulation by lymphocytes. The writer showed previously that the stroma in a transplanted LN preserved its donor origin [3], whereas the lymphocytes were of recipient origin. Nevertheless, the reticulum underwent considerable structural modification, as shown by mitoses of the stromal cells and the newly formed network of reticulin and collagen fibers.

Data on migration of ^{51}Cr-labeled cells and the results of the morphological observations revealed marked ability of lymphocytes of the peripheral pool to colonize the regenerating stroma. However, they migrated into the implant in much smaller numbers, although their level of migration significantly exceeded that into nonlymphoid organs such as the skin and muscle tissue. The newly formed reticulum, with its network of blood vessels and lymphatics, evidently provides the necessary microenvironment for homing of the circulating lymphocytes. One cause of the sharply reduced migration of lymphocytes into the implant is overpopulation with lymphocytes due to disturbance of the regular outflow of lymphocytes. Histological analysis of the bed of the implants, attached to the muscle wall, revealed chaotic outflowing of lymphocytes along small lymphatics (see Fig. 1a). Evidence of the outflow of lymphocytes and their longer retention in the implant is given by a decrease in the proliferative activity of lymphocytes of the implant, and by the increased number of degenerating cells and of macrophages phagocytosing cell debris in the sinuses, evidently due to the fact that lymphocytes repopulating the lymphoid organs are at different stages of development and differentiation. However, the possibility cannot be ruled out that a decrease in the number of lymphocytes entering the implant may be due also to a decrease in the total surface area of the postcapillary venules with high endothelium during regeneration.

We know that LN is formed on the basis of plexuses of lymphatics [4, 6, 7, 14], which are subsequently converted into sinuses of the gland. On the basis of the facts described above it can be postulated that the system of sinuses of LN, formed primarily when the anlage of the gland is laid down, plays a definite role in its structural organization and a leading role in the regulation of entry of lymphocytes into their departure from the lymph node.

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ERYTHROCYTE-DAMAGING AND IMMUNOMODULATING ACTION OF RETINOIDS

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UDC 615.356:577.161.11].065:616.151.1+
612.017.1.014.46:615.356:577.161.11

KEY WORDS: erythrocytes; retinoids; immunity.

The ability of vitamin A and structurally related compounds (retinoids) to stimulate immunity has been known for a relatively long time [5]. According to some reports, under the influence of compounds of this group both humoral and cellular immunity may be modified [6, 8, 9]. Attempts have been made to use vitamin A for immunostimulation in man [7]. However, the pathways of adjuvant action of vitamin A and retinoids have not yet been explained. The writers reported previously [1] that under the influence of vitamin A and retinoids lymphocyte formation is increased in the red bone marrow, the number of large and medium-sized lymphocytes in the blood and the number of small lymphoid follicles in the spleen are increased, and functional activity of the spleen cells is enhanced. Another investigation [4] showed that excessive doses of retinoids damage erythrocytes by reducing their osmotic resistance and stimulating erythropoiesis in the red bone marrow. These results served as the basis for the hypothesis that the adjuvant action of vitamin A and retinoids is mediated through damaged erythrocytes, which are expelled from the blood stream by an immunocellular mechanism [3].

The aim of this investigation was to study correlation between changes in the blood, splenic pulp, and functional activity of the liver macrophages of mice under the influence of retinoids.

EXPERIMENTAL METHOD

Experiments were carried out on adult mature mice of both sexes, either noninbred or pure-line CBA and (CBA × C57BL/6)F_1 (henceforward abbreviated to F_1) hybrid mice, weighing 16-20 g. The number of animals in each group, the doses and methods of administration of retinoic acid and its esterified cis- and trans-isomers, retinyl acetate (RA), and retinoids C_{15} and C_{20}, are given in Tables 1 and 2. The substances were obtained from the Laboratory of Chemistry of Polyenic Compounds (Head, Professor G. I. Samokhvalov), of the "Vitaminy" Research-Production Combine (USSR). The structures and trivial names of the compounds were given in [8]. The state of the erythrocytes was judged by the hemoglobin concentration in the blood, the number of erythrocytes in 1 mm^3 of blood, and the osmotic resistance of the erythrocytes. These parameters were determined as described previously [2]. The number of reticulocytes (in promille) was determined in blood films stained supravitally with bright cresyl blue.

To determine the delayed-type hypersensitivity reaction (DTHR) to purified tuberculin the animals were immunized by a single intraperitoneal injection of BCG vaccine in a dose of 0.5 ml of a 0.2% suspension in physiological saline. RA was injected intraperitoneally twice a week in a dose of 0.5 ml of a 0.2% oily solution. On the 21st day of the experiment (the optimal time for manifestation of the immunomodulating action of BCG vaccine and of vitamin A) 0.01 unit of purified tuberculin in 0.1 ml physiological saline was injected

Department of Histology, Cytology, and Embryology, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kovanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 10, pp. 463-466, October, 1985. Original article submitted October 8, 1984.

 0007-4888/85/0010-1398$09.50