Brief Definitive Report

T-CELL MIGRATION INTO ALLOGRAFTS*

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There is evidence that rejection of solid tissue allografts is initiated by T cells. In vitro, T lymphocytes can kill cells with transplantation antigens against which they have been sensitized (1). However, in vivo other cells may participate in graft destruction. In this regard, morphologic studies have shown several types of mononuclear cells in grafts, although large pyroninophilic cells, thought to be young lymphocytes, usually predominate early. A priori, one might conclude that the great majority of these are T cells, but this is not necessarily so, since a few T cells might trigger the influx of other cells. Recently, Balch et al. (2), employing fluoresceinated antisera to rat thymocytes, concluded that T cells predominate in early renal allografts. However, there are technical difficulties in identifying cell surface markers with certainty in tissue sections by immunofluorescence.

Another approach is to study the propensity of various populations of mononuclear cells to accumulate in graft infiltrates after transfer of labeled cells. Such methods have provided information about cells in delayed reactions (3). The development of techniques for the preparation of highly purified populations of T and B cells has greatly enhanced the value of this approach (4). The present study deals with the ability of purified populations of T and B lymphocytes to accumulate in skin grafts in mice.

Material and Methods

Animals. Male CBA/J, A/J, and C57BL/6 mice, 8-12 wk old, were obtained from Jackson Laboratories, Bar Harbor, Maine. CBA/J (H-2k) mice served as skin allograft recipients and A/J (H-2q) and C57BL/6 (H-2q) mice as donors.

Sensitization. CBA/J mice were sensitized against A/J or C57BL/6 mice by placing tail skin grafts on the thorax. 11 days later the mice were boosted by an intraperitoneal injection of 50 x 10^6 spleen cells from the same strain as the skin donors. 5 days later the mice were sacrificed and suspensions of spleen cells were prepared.

Immunoabsorbent Columns. Purified T- and B-cell populations were prepared as described previously (4). In brief, purified rabbit antimouse Fab was conjugated to Sephadex G200 by cyanogen bromide. A packed 12-ml Sephadex column was washed with 0.005 M EDTA, Liebowitz-15 (L-15), 5% fetal calf serum, and 50 U/ml penicillin-streptomycin. To the column was added 250 x 10^6 macrophage depleted spleen cells in column medium (4). The effluent (T) cell fraction was collected for 2 h at 4°C. The B cells were then eluted, using 10% autologous mouse serum as a source of mouse gammaglobulin (5).

Labeling of Cells. Spleen cell suspensions and purified populations of T and B cells were labeled

* This work was supported by grants CA-15679 and AM 16392-03 from the National Institutes of Health and by grant IC 48 from the American Cancer Society.
by incubation with 10 µCi of [3H]uridine (spec act 9.7 Ci/mmol, New England Nuclear Corp., Boston, Mass.) per ml for 50 min at 37°C. The cells were washed three times and suspended in medium 199 (Microbiological Associates, Inc., Bethesda, Md.).

In Vivo Migration. [3H]-uridine-labeled cells were injected into the tail vein of CBA mice, which 7 days previously had received skin isografts on the left flank and skin allografts, from A/J or C57BL/6 mice, on the right side. In some experiments, the mice had received both A/J and C57BL/6 skin grafts. The mice were sacrificed 24 h after transfer. The isografts, allografts, lymph nodes, spleen, and gut were fixed in 10% formalin, embedded in paraffin, and sectioned at 3-4 μm. Smears were prepared with the cytocentrifuge.

Autoradiographs were prepared with NTB-2 emulsion (Eastman Kodak Co., Rochester, N.Y.). The smears were exposed for 7 days and the sections for 5 wk. The sections were stained with hematoxylin and eosin and the smears by the Giemsa method. The percentage of labeled cells in the graft infiltrates was estimated by counting the number of labeled and unlabeled mononuclear cells in the areas of most intense infiltration.

Results

Exploratory studies showed that the allografts were rejected in 10-14 days. Mononuclear cell infiltration was found to be most intense at 7 days; therefore all transfer studies were performed at this time. The isografts showed only very scanty mononuclear infiltrates.

In the first experiments, labeled whole spleen cell suspensions (100 x 10⁶), were injected into CBA/J mice with allografts from A/J or C57BL/6 mice. More than 85% of the donor cells were labeled and all of the large lymphocytes (> 12 μm in diameter), which formed 12-14% of the population, were heavily labeled. In the recipients, numerous labeled cells were found in lymph nodes and lymphoid tissue of the spleen and intestine. In addition, labeled cells were seen in large numbers in the allografts, especially in the infiltrate at the graft margins where they averaged about 10% of the infiltrating cells. A few labeled cells were also seen in the epidermis of the allografts, near the lateral margins. Only a very few labeled cells were seen in the isografts.

Similar experiments were then performed with purified populations of T and B lymphocytes. On average, the total recovery (i.e. of both T and B cells) from the column was 90% or greater. Fewer than 5% of the cells passing through the column (T-cell population) showed granular surface staining for mouse Ig, whereas 95-100% of the cells eluted from the column (B-cell population) showed such staining. After incubation with [3H]uridine more than 90% of the B-cell population and more than 80% of the T-cell population were labeled. Large lymphocytes, which formed 12-14% of the T- and B-cell suspensions, were most heavily labeled.

Autoradiographs of recipients of labeled T or B cells (25 x 10⁶ cells) showed that both cell populations had homed to lymphoid tissue in large numbers; the T-cell population to T-cell areas and the B-cell population to both T- and B-cell areas. Table I summarizes the findings in the grafts. In all experiments, after transfer of T lymphocytes, labeled cells were readily found in allografts in numbers comparable to those seen after injection of whole spleen cell suspensions. Labeled cells were found in the cellular infiltrate in the dermis (Fig. 1), and in smaller numbers in the epidermis (Fig. 2). In striking contrast, after transfer of B cells, almost no labeled cells were found in the infiltrate around the allograft and none in the epidermis. In isografts, only extremely rare labeled cells were seen after transfer either of labeled T or B cells.
**Table I**

**Accumulation of Labeled Cells in Skin Grafts 24 h after Intravenous Injection of Unfractionated Spleen Cells and Purified Populations of T and B Lymphocytes Labeled in Vitro with $[^3H]$Uridine***

| Exp. | Cell donors (CBA/J) sensitized against | No. of recipients | Cell type | Ig-bearing cells | Percentage of labeled cells in the cellular infiltrate | A/J allograft | Isograft | C57BL/6 allograft |
|------|----------------------------------------|-------------------|-----------|-----------------|-----------------------------------------------------|--------------|---------|------------------|
| 1    | A/J                                    | 3                 | T         | 1               | %                                                   | %            | %       | Rare             |
|      |                                        | 2                 | B         | 95              | 0                                                   | 0            | ND      | ND               |
| 2    | A/J                                    | 3                 | Sp§       | 55              | 7                                                   | Rare         | Rare    | ND               |
|      |                                        | 4                 | T         | 2               | 11                                                  | 0            | ND      | ND               |
|      |                                        | 4                 | B         | 96              | Rare                                                | 0            | ND      | ND               |
| 3    | A/J                                    | 4                 | Sp        | 59              | 9                                                   | Rare         | Rare    | ND               |
|      |                                        | 3                 | T         | 4               | 8                                                   | 0            | ND      | ND               |
|      |                                        | 4                 | B         | 100             | 1                                                   | Rare         | ND      | ND               |
| 4    | A/J                                    | 3                 | Sp†       | 55              | 6                                                   | ND           | 1       |                  |
|      |                                        | 3                 | T         | 5               | 7                                                   | ND           | 3       |                  |
|      |                                        | 3                 | B         | 96              | Rare                                                | ND           | 0       |                  |
| 5    | C57BL/6                                 | 4                 | Sp*       | 40              | 7                                                   | ND           | 10      |                  |
|      |                                        | 2                 | T         | 4               | 5                                                   | ND           | 8       |                  |
|      |                                        | 3                 | B         | 95              | Rare                                                | ND           | Rare    | Rare             |

* Number of cells injected into each recipient: spleen cells, $50 \times 10^6$; T lymphocytes, $25 \times 10^6$; B lymphocytes, $25 \times 10^6$ cells.
† The percentage of labeled cells was estimated by counting in autoradiographs the number of labeled cells among cells judged to be infiltrating cells in the area of maximum cellular infiltration. The total number of cells counted in each autoradiograph ranged from 100–200.
§ Sp, Spleen cells depleted of macrophages.
†† Each recipient also received unlabeled spleen cells ($50 \times 10^6$) from CBA/J mice sensitized to C57BL/6.
* Each recipient also received unlabeled spleen cells ($50 \times 10^6$) from CBA/J mice sensitized to A/J.

In some experiments, labeled T lymphocytes from mice sensitized to either A/J or C57BL/6 antigens were injected into CBA mice with skin grafts from both A/J and C57BL/6 mice. Considerable numbers of labeled cells were seen in both allografts and showed similar distribution in each.

**Discussion**

In the present study it was found that after transfer of purified populations of labeled T lymphocytes or whole spleen cell populations, large numbers of labeled cells accumulated in recently transplanted skin allografts, but not in isografts. In some areas, labeled cells represented about 10% of the mononuclear cells; since they were diluted by host cells these findings indicate a striking propensity of the T-cell population to accumulate in allografts. In contrast, after transfer of purified populations of B cells, only an extremely small number of labeled cells were found in the grafts. That this did not result from damage inflicted on the cells during purification is indicated by the fact that the B-cell populations homed to lymphoid tissue, reflecting normal physiological migration properties. Further, in vitro studies of cells recovered from columns have shown them to be functionally intact (5, 6).

In view of the high degree of purity of the T-cell population, it seems probable
Fig. 1. Autoradiograph of section of 8-day old A/J skin allograft 24 h after transfer of \(^3\)H-uridine-labeled T lymphocytes from CBA/J-sensitized donors. Labeled cells are seen in the cellular infiltrate of the dermis of the graft. (hematoxylin and eosin, × 400).

Fig. 2. Higher magnification of the allograft in Fig. 1 to show labeled T lymphocytes in the epidermis of the graft. (hematoxylin and eosin × 1,000).
that most, if not nearly all of the labeled cells in the grafts were, in fact, T cells rather than some contaminating cells. There were few monocytes in the T-cell preparation, as judged by morphological criteria. There are, however, significant percentages of cells that are not stained with antimouse brain serum or killed by anti-theta serum and complement (4). This population, referred to as the null cell population, may include immature B cells, T cells with little or no theta antigen, or other unclassifiable mononuclear cells. The possibility that some of the null cell population migrated to the skin allografts cannot be excluded.

Another consideration is that only 7-day grafts were studied; possibly at later times larger numbers of immunoglobulin-bearing B cells might accumulate.

Previous studies using transfer of labeled whole spleen or lymph node suspensions have shown that certain newly formed lymphocytes have the capacity to accumulate in cell-mediated reactions and in certain other inflammatory reactions (3). The present studies show that at least the majority of such cells are T lymphocytes.

These studies were not designed to examine the possibility that T cells specifically sensitized to antigens in a particular graft show a greater tendency to accumulate in that graft than in an unrelated graft. However, in a few mice in which two types of allografts were present, cells from donors sensitized against only one graft found their way into both grafts, in what appeared to be comparable numbers. Most previous studies have failed to detect preferential accumulation of sensitized cells in cell-mediated reactions, although in some, preferential accumulation has been claimed (3). In any case, it is clear from the present study that T cells can accumulate in a reaction initiated by antigens against which the cells were not specifically sensitized.

Summary

The ability of T and B lymphocytes to migrate into skin allografts undergoing rejection was studied in mice. Spleen cells from CBA/J mice sensitized to transplantation antigens of A/J or C57BL/6 mice were separated on immunoabsorbent columns into purified populations of T and B cells, labeled in vitro with ³H-uridine and injected intravenously into CBA/J mice with 7-day old skin isografts (A/J or C57BL/6). The mice were sacrificed 24 h later and studied by autoradiography. After transfer of either unfractionated spleen cells or T cells, large numbers of labeled cells were found in the cellular infiltrate of allografts, whereas extremely few were seen in isografts. In contrast, after transfer of B cells, almost no labeled cells were detected either in the allografts or the isografts, although they, like T cells, homed normally to lymphoid tissue.

Received for publication 20 January 1975.

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