TRANSFER OF CYTOTOXIC ACTIVITY OF LYMPHOCYTES IMMUNE TO *Mycobacterium tuberculosis*

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Hypersensitivity of delayed type (HDT) was transferred in syngeneic, allogeneic, and semisyngeneic systems with the aid of lymphocytes taken from donors vaccinated with BCG. HDT was tested by means of the cytotoxic effect (CE) in which the effector cells were lymphocytes of recipients while the target cells were antigen-containing peritoneal macrophages. With syngeneic transfer the CE was detectable after 1 day and could be tested until the end of the experiment (30th day), while with allogeneic transfer (CBA→A) the CE fell below the level of reliable detection by the 14th day. With semisyngeneic transfer (CBA → F_1 hybrids) inactivation of the recipient cells by antilymphocytic serum (CBA anti-A) did not change the CE immediately after transfer but reduced it sharply in later periods. It is postulated that during transfer sensitization is "induced" on the recipient's cells.

The main problem arising in the study of the transfer of hypersensitivity of delayed type (HDT) is whether the donor's cells themselves reject the homograft, accumulate in tuberculin skin tests, and so on, or whether they somehow "induce" a state of sensitization on the recipient's cells. Until recently the main method used to assess HDT was by skin tests, and this greatly restricted the scope of such investigations.

In work to study transferred HDT contradictory results have been obtained. Some workers have reported that mainly donor's cells accumulate in tuberculin skin tests and infiltrate the graft on recipient animals [16], while others have shown [12, 15] that the number of donor's cells in such reactions is negligible. However, it must be noted that a few sensitized donor's cells can mobilize the entire cell population [7].

During the last decade a series of tests enabling HDT to be studied in vitro has been developed; in these tests effector cells of HDT interact with sensitizing antigen [9, 17, 22].

The method of cytotoxic action of lymphocytes on target cells, widely used to study transplantation and antitumor immunity and autoimmune responses [1, 5, 18, 22], has been modified in the writers' laboratory. In this modification sensitized lymphocytes interact with target cells containing mycobacterial antigens on their surface [3].

The object of this investigation was to use the method described above in order to study whether HDT can be transferred and the role of the donor's and recipient's cells in transferred HDT.

EXPERIMENTAL METHOD

Experiments were carried out on 752 CBA, A, and (CBA × A)F_1 hybrid mice. The CBA mice were immunized with living BCG vaccine (1 mg), the lymph glands were taken from the animals one month later, and a suspension was prepared from them for injection into intact recipients. Each recipient received 25 million cells intravenously and intraperitoneally. The transfer was carried out on syngeneic (CBA → CBA), allogeneic (CBA→A), and semisyngeneic (CBA → F_1) systems (Fig. 1). At different times after the transfer lymph glands were taken from the recipients of the lymphocytes, and the activity of the lymphocytes was in-

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TABLE 1. Cytotoxic Action of Recipients' Lymphocytes on Antigen-Containing Target Cells after Transfer of Lymphocytes from Donors Vaccinated with BCG (M ± m)

Donor → recipient	Time after transfer (in days)					
	1	3	7	14	18	30
CBA→CBA	24,4±3,1*	34,4±3,2*	51,6±3,2*	58,6±2,9*	—	31,3±3,2*
CBA→A	—	29,4±3,2*	—	5,2±1,6†	—	—
CBA→F_1 (CBA×A)	—	47,5±2,9* / 49,7±2,9*	—	—	55,2±3,0* / 10,7±2,2†	

* $P<0,02$.
† $P<0,05$.

Note. Numerator – after treatment with normal CBA serum; denominator – after treatment with CBA-anti-A immune serum.

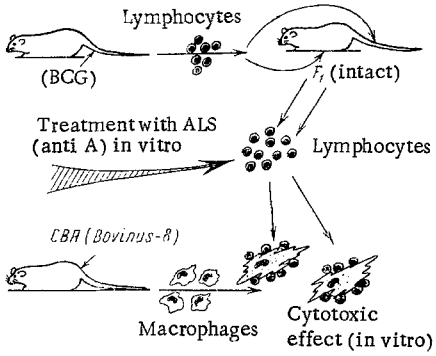

Fig. 1. Schematic layout for the transfer in a semisyngeneic system.

vestigated by the cytotoxic test [4]. Peritoneal macrophages (4-day exudate after injection of thioglycol broth) of animals infected with Mycobacterium tuberculosis strain Bovinus-8 (0.1 mg) or of control animals were used as the target cells. The macrophages were grown in flat-bottomed tubes for 48 h in medium No. 199 with 20% bovine serum and 10-15% lactalbumin, (total volume 1 ml) in a density of 200,000 cells/ml. After 48 h, when the macrophages were adherent to the bottom of the tube and spread out, the supernatant was poured off and lymphocytes were added in the ratio of 100 viable lymphocytes per macrophage in medium No. 199 with 15% lactalbumin. After 2 days the medium was replaced with fresh (with 2% serum), and after a further 24 h the results were read. The cytotoxic index

$$I = \frac{a-b}{a},$$

was calculated, where a is the number of viable macrophages in the control (after addition of lymphocytes from recipients of normal lymphocytes), and b is the corresponding number in the experiment (i.e., using lymphocytes from animals receiving immune cells). Viability was assessed by staining with trypan blue. The method of discriminative analysis [6] was used in the experiment in the semisyngeneic system. Before addition of lymphocytes of passively sensitized F_1 hybrids to the macrophages, the lymphocytes were treated for 1 h at 37°C with CBA anti-A antilymphocytic serum and complement (in the proportion of 20 million lymphocytes to 0.1 ml serum and 0.1 ml complement in medium No. 199 in a total volume of 1 ml. After treatment the lymphocytes were washed twice and added to the macrophages in the same proportion as in the other experiments [counting only viable lymphocytes (Fig. 1)]. The serum was obtained by immunization four times by the following scheme. Lymphocytes from the lymph glands of A mice were injected into CDA mice at the rate of one A mouse → 5 CBA mice subcutaneously, followed one week later by 1A → 3CBA intraperitoneally, one month later by 1A → 3CBA intraperitoneally, and another week later by 1A → 3CBA intraperitoneally. Serum was obtained after one week, and its effectiveness was tested by the method described by Govallo et al. [2]. The index of the anti-A and anti-F_1 antilymphocytic serum was 0.4.

EXPERIMENTAL RESULTS

Where lymphocytes of vaccinated CBA mice were transferred to mice of the same line (Table 1), the lymphocytes of the recipients had a cytotoxic action on the target cells after 24 h. This cytotoxic activity rose to a maximum after 14 days and then began to fall, but even after 30 days the recipients' lymphocytes still had a cytotoxic action.

In allogeneic transfer of sensitized cells induction of cytotoxicity also took place three days after transfer, although when the cytotoxic effect was tested after 14 days it was weak or absent altogether.

With semisyngeneic transfer the recipient's lymphocytes also had a cytotoxic action on antigen-containing macrophages from CBA mice (when tested 3 and 18 days after transfer). If, however, before addition to the culture of macrophages, the lymphocytes were treated with antilymphocytic serum (CBA anti-A), which when mixed with cells of the F_1 hybrid recipient and the CBA donor should inactive only the cells of the (CBA × A) hybrid, the cytotoxic effect was almost unchanged after 3 days, but after 18 days it was sharply reduced. Treatment with normal (CBA) serum had no effect on the cytotoxic action of the lymphocytes.

In these experiments macrophages from infected CBA mice were used as the target cells in order to avoid any possible reaction of the transferred lymphocytes of the CBA mice against antigens of the line A mice, if A or F_1 macrophages were used as the target cells. It was also found that lymphocytes obtained from the F_1 hybrid recipients of sensitized cells had no cytotoxic action if intact (not containing antigen) CBA macrophages were used as the target cells.

These investigations thus showed that transfer of HDT, tested by the cytotoxic action of the lymphocytes on antigen-containing target cells, can be transferred by means of lymphocytes of actively sensitized donors in mice. The cytotoxic effect can be transferred in syngeneic, allogeneic and semisyngeneic systems. Cytotoxicity of the lymphocytes can be connected with their interaction with antibodies on target cells [18], but when intact macrophages of hyperimmune (against mycobacterial antigens) rabbit serum or the serum of mice vaccinated with BCG were used, it was impossible to induce cytotoxicity of the lymphocytes of BCG-vaccinated donors.

In the case of rapid rejection of the cells (as in allogeneic transfer), the cytotoxic effect was not "induced" and disappeared. This shows that soon after transfer the cytotoxic effect is produced by the donor's cells themselves.

Experiments in a semisyngeneic system showed that during inactivation of the recipient's cells by antilymphocytic serum the cytotoxic effect was unchanged soon after transfer but fell sharply in the later stages. It is considered that these experiments demonstrate the induction of cytotoxicity on the recipient's lymphocytes which were inactivated by the antilymphocytic serum, although active immunization cannot be ruled out completely. Admittedly, the unsuccessful attempts to find antigen in the recipient's organs (seeding the organs to detect the presence of mycobacteria, staining by Ziehl's or immunofluorescence methods) and also the disappearance of cytotoxicity after rejection of the donor's cells in the case of allogeneic transfer are to some extent evidence against active immunization.

The hypothetical process of induction is possibly analogous to that taking place when sensitized lymphocytes secrete factors reproducing certain manifestations of HDT in vitro [8, 10, 11, 13, 19, 20, 23]. In infectious pathology it would be extremely interesting to understand how and to what degree sensitization can be "induced" in a focus of inflammation and how this affects the course of the specific process.

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