Reprinted from Immunology Today – *September, 1982* Vol. 3, No. 9, pp. 250–252

T-cell recruitment: a tool for specific immunosuppression

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The induction of selective unresponsiveness in a graft recipient towards the foreign antigens of the graft is a prime goal of transplantation immunology.

Today, with the increasing use of bone-marrow transplantation and the associated threat of graft v. host (GVH) disease, it is of utmost importance to reduce in the donor-cell population the numbers or activities of competent lymphocytes potentially reactive against histocompatibility antigens of the recipient. However, specific immunosuppression has not yet been realized clinically and all procedures currently in use involve pharmacological suppression of the immune apparatus of the donor or the recipient, too often resulting in serious and harmful side effects[1].

In experimental systems, specific immunosuppression has been obtained by selective recruitment of specific lymphocytes from the circulating pool to particular lymphoid organs such as spleen, bone marrow and liver, by injecting animals intravenously with allogeneic cells[2,3].

We have developed a peripheral sensitization model of specific immunosuppression[4,5]. In our system the donor is inoculated with his own T lymphocytes that have been sensitized against allogeneic antigen *in vitro*. We have found that injection with these peripherally sensitized lymphocytes, called initiator T lymphocytes (T_I) leads to trapping and sequestration of effector T lymphocytes (T_E) in a local lymph node. Recruitment of T_E to the draining popliteal lymph node (PLN) by T_I depleted the specific GVH potential of the populations of cells in the spleen or thoracic duct in a model of bone marrow transplantation in mice[6]. Furthermore, excision of the lymph node containing recruited T_E rendered the mice specifically immunosuppressed and increased significantly the survival of a specific allogeneic skin graft[7].

The recruitment system

Our general approach has been to separate the primary triggering event of the immune response, the initial contact of lymphocytes with immunogen, from the afferent flow of information to the lymph node and the central generation of effector lymphocytes. This was accomplished by inducing sensitization of naive populations of T_I against allogeneic antigens *in vitro* for short periods of time. The sensitized T_I were then separated from the alloantigen (fibroblasts or macrophages), irradiated to prevent further proliferation or differentiation and injected into the foot pads of syngeneic mice. These peripherally sensitized T_I did not appear able to differentiate into effector cells[8]. Rather, they were found to migrate within 12–16 h to the draining PLN and there to signal an immunospecific trapping of potential T_E from the general pool of cells recirculating through the lymph nodes. T_I, in contrast to T_E, do not appear to recirculate[9]. The kinetics of T_E trapping paralleled those of PLN enlargement. Recruitment began about 3 days after injection of the T_I, reached a maximum at 6 days, and declined to control values by about 8–10 days (Fig. 1). The recruited lymphocytes gave rise to immunospecific cytotoxic T_E, which preferentially lyse specific targets, and to memory cells, identified as new T_I, which could be resensitized to allogeneic sensitizing cells of the same H-2 type as the original cells[10]. The spleen and distal nodes were found to be depleted transiently of effector lymphocytes capable of reacting against the same allogeneic antigens to which the T_I originally had been sensitized. The kinetics of this depletion from distal lymphoid organs correlated with the kinetics of the lymphocyte trapping in the PLN during recruitment (Fig. 1). Cytotoxic T lymphocytes were detectable in the PLN at the peak of T_E trapping[10].

The recruitment model enabled us to manipulate the complement of potential effector cells of a given specificity. We recruited specific effector lymphocytes

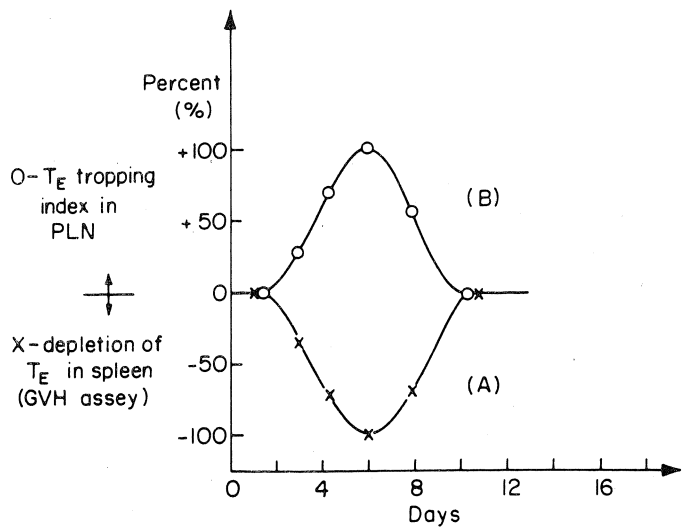

Fig. 1. Kinetics of T_E trapping and depletion in recruitment

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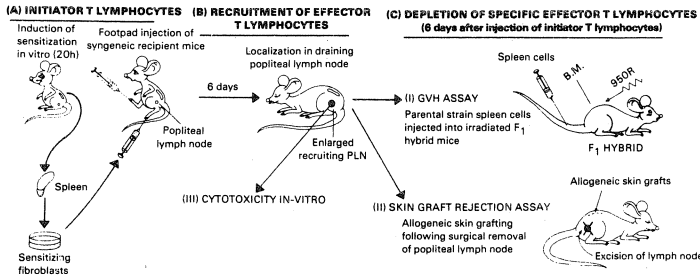

Fig. 2. Outline of recruitment experiments (see text).

to the popliteal lymph node of mice and then used those mice either as:

(1) donors of spleen cells in a GVH assay or,

(2) as recipients of skin allografts after the popliteal lymph nodes were excised.

Fig. 2 describes the basic design of these experiments.

Depletion of GVH-reactive lymphocytes from the spleen

The GVH potential was tested in a systemic, quantitative and lethal parent to F_1 system [C57BL/6 → (C3HeB × C57BL/6)F_1; C57BL/6 → (BALB/c × C57BL/6)F_1]. We used two different assays: (1) *Sublethal assay*; parental strain donor spleen cells, 50×10^6, were injected intravenously into F_1 recipients that had been sublethally irradiated (450 R). (2) *Lethal assay;* parental strain donor spleen cells, usually 5×10^6, together with 2×10^6 F_1 bone marrow cells, were injected intravenously into F_1 mice that had been lethally irradiated (950 R).

We found that recruitment to a draining lymph node caused a 2–5-fold depletion of the immunospecific GVH potential of lymphocyte populations in the spleen[5] and in the thoracic duct[6].

Allogeneic fibroblasts contaminating the T_I do not cause recruitment

Experiments were also done to exclude the possibility that allogeneic fibroblasts or alloantigen in some other form were not at least partly responsible for recruitment rather than syngeneic T_I cells. We found that 10^5 or 10^7 allogeneic fibroblasts caused no detectable effect on day 6, the time of most marked depletion of specific reactivity following injection of sensitized T_I (Ref. 5). Thus, injection of up to 200-fold more allogeneic fibroblasts than the number contaminating the T_I population could not induce depletion of GVH lymphocytes.

It seems therefore that the recruitable GVH-reactive lymphocytes are induced to be trapped by sensitized syngeneic T_I. Antigen by itself did not produce the depletion we observed.

Depletion of effector lymphocytes rejecting an allogeneic skin graft

In order to confirm the immunospecific recruitment of effector lymphocytes to the regional lymph node, we excised the recruiting PLN at the height of T-lymphocyte trapping and transplanted the depleted mice with allogeneic skin grafts, using the skin grafting technique developed in our laboratory[11]. We found that excision of the recipient's PLN at the peak of recruitment prolonged the survival time of skin allografts from 11.9 ± 0.5 days in the control group to 22.1 ± 2.1 days in the specifically depleted recipient mice[7]. Thus, the PLN recruited a significant number of potential effector T lymphocytes committed to the allogeneic *H-2* skin graft.

T_I and T_E belong to different sets of T cells

Table I summarizes characteristics of T_I and T_E. It can be seen that the cells are distinct with regard to their Ly phenotype, their organ of residence and their migratory behavior.

TABLE I. Characteristics of T_I and T_E

	T_I	T_E
Lifespan	Short	Long
Recirculation	No	Yes
Activity in organs		
Spleen	+	+
Thymus	+	–
Lymph node	–	+
Thoracic duct	–	+
Found in B mice	No	No
Thy-1 phenotype	Positive	Positive
Ly phenotype	Ly-1+2–	Ly1–2+
Effect of adult thymectomy	Sensitive	Resistant
Effect of antithymocyte antiserum	Resistant	Sensitive
Irradiation	Resistant	Sensitive

A model of T-cell recruitment

The experiments discussed above have established several principles. First, the recruitment response

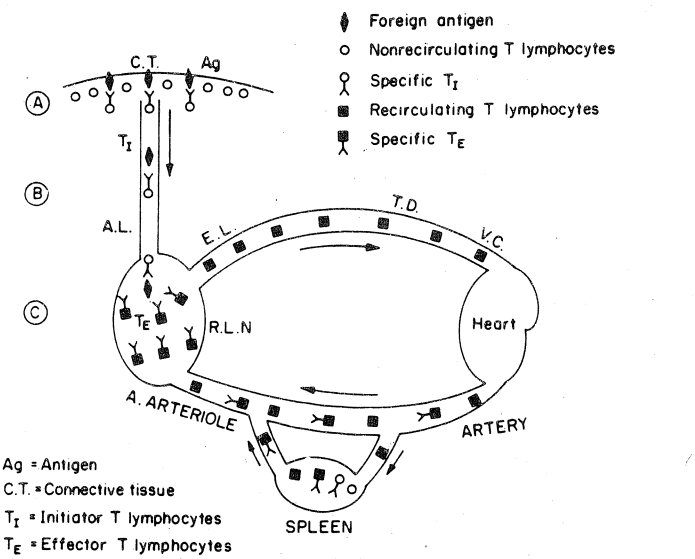

Fig. 3. Model of recruitment *in vivo* (see text).

involves sequential interactions between different types of cells: sensitizing cells (antigen) — T_I — T_E. Second, recruitment to a local lymph node is immunospecific, leading to transient depletion of effector lymphocytes from the spleen and other lymphoid organs. Third, it is possible to achieve specific immunosuppression in models of bone marrow and allograft transplantation by exploiting the physiological redistribution of immunospecific lymphocytes that occurs during the recruitment reaction. We are currently investigating the cellular mechanisms involved in this immunosuppression. The finding of cytotoxic T lymphocytes in the draining lymph node along with the reduction of specific T_E function in other lymphoid organs indicates that T_E may be physically sequestered in the lymph node. However, recent work (Belldegrün, A. and Cohen, I. R., in preparation), using mixtures of cells from various lymphoid sources, raises the possibility that the specific immunosuppression mediated by recruitment is caused, at least in part, by active suppression, in addition to trapping of specific lymphocytes in a particular lymph node. On the basis of the recruitment model (Fig. 3), it is conceivable that T_I might function *in vivo* to patrol the tissues and recognize invading immunogens. Following specific sensitization the receptor-bearing T_I migrate to the lymph nodes and there they signal another type of T lymphocyte, circulating T_E, to differentiate to immunospecific effector T lymphocytes. The latter, then, constitute the efferent phase of the reaction.

Do the T_I function merely by presenting the antigen to the T_E? It was observed by Livnat and Cohen that trypsinization of the T_I prevented their capacity to recruit[12]. Yet 4 h after trypsinization the T_I regained, in the absence of antigen, the capacity to recruit effectors.

On the basis of work done by Lafferty, Bach and others[13,14], together with our results, it seems that T_I–T_E interaction involves a two-signal process requiring presentation of both antigen and an inductive molecule by sensitized T_I to the recruited T_E. Thus, recruitment occurs when the T_E binds antigen through its surface receptor and simultaneously receives an inductive stimulus from the sensitized stimulator T_I cell, which also seems to carry a receptor for antigen on its surface. The delivery of both signals results in activation to T_E.

An increased understanding of the cell migration and interactions which take place in the generation of cell-mediated immunity should lead to new approaches toward selective manipulation of the immune response for clinical objectives.

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