# Autosensitization of Lymphocytes against Thymus Reticulum Cells

Abstract. *Lymph nodes of normal rats contain lymphocytes that can be induced in vitro to mediate a specific cellular immune reaction against reticulum cells derived from syngeneic adult thymus glands. It is likely that these lymphocytes had access to reticulum cell antigens during the period in which they developed immunocompetence within the thymus. This suggests that contact with self-antigens during development may not eliminate self-reactive lymphocytes. These findings are in conflict with the theory that natural tolerance to self-antigens depends upon elimination of lymphocyte clones*

The healthy immune system usually responds to foreign antigens, but not to the body's own antigens; nonreactivity to self is a basic concept of immunology. The breakdown of natural tolerance and the activation of the immune system against self-antigens can produce an autoimmune disease (*1*). Hence, the mechanisms that ensure tolerance to self are likely to have evolved as an integral part of the immune system. However, the cellular basis of natural self-tolerance has not been easy to study directly.

According to Burnet's clonal selection theory (*2*), natural tolerance to self-antigens is based on the active elimination of clones of potentially self-reactive lymphocytes during a critical period in the maturation of the immune system. Lymphocytes that bear receptors for specific antigens are killed by contact with their complementary antigens in this developmental period. Therefore, normal exposure to self-antigens, as well as abnormal exposure to foreign antigens, leads to the elimination of reactive lymphocytes, and to subsequent tolerance. Exposure to other antigens after the critical period stimulates a typical immune response.

Jerne (*3*) has recently attempted to apply the concept of lymphocyte elimination in order to explain the diversity of specific antibodies or effector lymphocytes produced by the immune system. Jerne's theory is that this diversity is generated by the somatic mutation of a few original immune system genes carried by the germ cells. Some of the original genes code for immune reactivity against the animal's own histocompatability antigens; however, such lymphocyte precursors that do not mutate will interact with self-antigens and be eliminated.

Lymphocytes that are derived from the thymus (T lymphocytes) play a direct role in cell-mediated immune reactions and cooperate in the induction of antibody production against a variety of antigens (*4*). Thymus reticulum cells provide an environment in which stem cells differentiate into immunocompetent lymphocytes (*5*). Only a very few of the large numbers of lymphocytes generated in the thymus ever leave to populate the peripheral lymphoid organs and to perform immune functions as T lymphocytes. Hence, Jerne suggested that the thymus may be an organ in which elimination of self-reactive lymphocytes generates the diversity of antibodies and effector lmphocytes and ensures the tolerance to self, both of which characterize the immune response (*3*). According to the Burnet and Jerne theories of elimination then, a normal animal could not have peripheral T lymphocytes that are potentially reactive against antigens in his own thymus.

To test this corollary experimentally, we studied the induction of autosensitization of lymph node lymphocytes of the rat against syngeneic thymus reticulum cell antigens in vitro. We report here that normal rats have lymphocytes that are capable of executing a cellular immune reaction against thymus reticulum cells.

We used an in vitro model of a cellular immune reaction (*6*), where lymph node cells are cultured on monolayers of sensitizing cells in modified Eagle's medium containing 15 percent horse serum. Lymphocytes with receptors that are complementary to monolayer antigens adhere to the monolayer cells (*7*) and, over a period of 5 days, are induced to replicate and transform into large sensitized lymphocytes. The sensitized lymphocytes are washed to separate them from the sensitizing cells and are then transferred to target cell cultures that have been labeled with $^{51}Cr$ introduced as sodium dichromate. The presence of specific sensitizing antigens activates the sensitized lymphocytes to damage the target cells by direct contact during 20 to 40 hours of culture. The percentage of the total $^{51}Cr$ label released from the target cells is then a measure of the lysis caused by the sensitized lymphocytes.

Lymph node cells from inbred Lewis rats (*8*) were autosensitized against adult Lewis syngeneic thymus reticulum cells, or against fibroblasts derived from 16- to 17-day-old Lewis rat embryos. Thymus reticulum cells were cultured in monolayers (*9*), and were identified by histochemical and electron microscopic studies. The degree of autosensitization of the lymphocytes was measured by their cytotoxic effects against cultures of syngeneic thymus reticulum cells, and syngeneic fibroblasts or foreign mouse fibroblasts (or both types of fibroblasts). Ten experiments were performed and all produced results similar to those of the four representative experiments shown in Table 1.

Thymus reticulum cells were lysed by lymphocytes that had been sensitized in vitro against either syngeneic thymus reticulum cells or embryonic fibroblasts. Syngeneic fibroblasts were usually lysed to a lesser degree than were thymus reticulum cells. The lysis of syngeneic target cells by autosensitized lymphocytes was usually at least three times greater than that of foreign mouse cells in any particular experiment. However, rat lymphocytes sensitized against mouse fibroblasts produced the reciprocal effect and lysed mouse target cells to a

Table 1. Sensitization and cytotoxic effects of lymph node cells from Lewis rats against Lewis thymus reticulum cells, or Lewis or mouse embryonic fibroblasts. Lewis lymph node cells ($30 \times 10^6$ to $40 \times 10^6$ cells) were sensitized for 5 days against $2 \times 10^6$ thymus cells or fibroblasts. Lysis was measured as the percent of $^{51}Cr$ released from $0.5 \times 10^6$ target cells by sensitized lymphocytes. The results are given as means of triplicate cultures, and are corrected for the spontaneous release of $^{51}Cr$ from control target cultures. The standard deviations of the means were all less than 1.5.

| Sensitizing cells | Target cell lysis (%) | | |
|---|---|---|---|
| | Lewis thymus reticulum | Lewis fibroblasts | C3H mouse fibroblasts |
| Lewis thymus reticulum* | 33 | 17 | 0 |
| Lewis fibroblasts† | 24 | 12 | 4 |
| C3H mouse fibroblasts* | 0 | 5 | 37 |
| C3H mouse fibroblasts† | 10 | 0 | 45 |

* Lymphocytes ($4.5 \times 10^6$ cells) were incubated with the target cell cultures for 40 hours. † Lymphocytes ($3 \times 10^6$ cells) were incubated with the target cell cultures for 24 hours.

greater degree than they did syngeneic rat cells (Table 1). These results indicate that a specific reaction of lymphocytes against syngeneic cells was induced in vitro. Thus, normal lymph nodes in the adult rat seem to contain T lymphocytes capable of mediating a cellular immune reaction against self-antigens present in the thymus. The self-antigens against which autosensitization was induced in cell culture remain to be identified. It appears that the same, or cross-reactive, antigens were present in both thymus reticulum cell and syngeneic fibroblast cultures.

We previously reported that rat or mouse lymphocytes autosensitized against syngeneic embryonic fibroblasts in vitro could produce graft-versus-host reactions in vivo (*10*). However, we could not rule out the possibility that sensitization had been induced against embryonic antigens that were not accessible to the developing immune system. Our findings here would seem to reduce this as a possibility. It is very unlikely that self-antigens in the thymus could have been sequestered from the developing cellular immune system because the lymphocytes that mediated cytoxicity also originated in the thymus (*11*).

The specificity of the cytotoxic effect against syngeneic target cells indicates that lysis was not the result of sensitization against extraneous foreign antigens present in the culture medium. There is a possibility that an undetected virus, or the in vitro culture conditions themselves, may have modified self-antigens to make them appear foreign to the lymphocytes. However, this is unlikely because lymphocytes autosensitized in vitro against fibroblasts (*10*) or thymus reticulum cells (*9*) can produce a specific graft-versus-host reaction in vivo.

We therefore conclude that elimination of lymphocytes cannot be the basis for natural tolerance to all self-antigens. Physiologic mechanisms that regulate the differentiation of immunocompetent lymphocytes are likely to function in the whole animal to prevent autosensitization and autoimmune disease. Our results imply that these regulatory mechanisms fail to operate in cell culture. Hence, potentially self-reactive lymphocytes may be induced in vitro to transform, replicate, and attack specific syngeneic antigens.

We have no direct evidence indicating the nature of the regulatory mechanisms that might function to maintain self-tolerance in vivo. However, specific regulation of the potential immune reactivity of lymphocytes against foreign antigens has been described in other systems, and natural tolerance to syngeneic antigens may result from similar processes.

Wegmann, Hellström, and Hellström found that lymphocytes from tetraparental (allophenic) mice were able to react against parental strain fibroblasts in vitro (*12*). These mice were formed by fusing unrelated mouse embryos at the eight-cell stage, well before the differentiation of the immune system. Reciprocal attack in vivo by the incompatible lymphocytes of these tetraparental mice appeared to be prevented by the presence of enhancing factors in their serum. Wegmann *et al.* suggested that the serum factors might be antibodies to parental strain antigens, or antigen-antibody complexes that blocked the activity of sensitized lymphocytes against mutually foreign parental antigens. However, in our system, preliminary incubation of sensitizing or target fibroblasts with normal Lewis rat serum did not inhibit either autosensitization or cytotoxicity (*13*). Therefore, enhancing antibodies probably do not maintain natural tolerance in vivo to the self-antigens to which they are reactive in vitro.

The immune response of animals to a foreign antigen may be paralyzed by injecting them with an excess amount of the specific antigen (*14*). However, lymphocytes that bind specific foreign antigens could be found after the induction of tolerance to these antigens (*15*), an indication that such lymphocytes may persist during a state of tolerance. Indirect evidence suggests that tolerant lymphocytes may be activated under certain conditions (*16*). It is possible, therefore, that an excess of self-antigen inhibits autosensitization in vivo in the same way that an excess of a foreign antigen can induce tolerance. Other findings indicate that tolerant lymphocytes may induce normal lymphocytes to be specifically tolerant of foreign antigens (*17*). Tolerance to self-antigens might also depend upon the function of specific inhibitory lymphocytes. It has not yet been determined if soluble antigens or tolerant lymphocytes can inhibit autosensitization in our system.

Burnet proposed two mechanisms compatible with the elimination theory to explain the breakdown of natural tolerance to self-antigens (*2*): (i) competent lymphocytes might react against self-antigens that were not available during the critical period of lymphocyte development; and (ii) somatic mutation of peripheral lymphocytes might produce "forbidden" clones reactive against self-antigens. However, neither of these mechanisms can easily explain our finding that normal adult rats possess competent peripheral lymphocytes that can be induced to react specifically against syngeneic thymus reticulum cells in vitro. The thymus antigens were probably accessible in vivo to the developing lymphocytes that mediated the cellular immune response in vitro. It is also unlikely that somatic mutation of lymphocytes occurred consistently in vitro to produce self-reactive "forbidden" clones. Hence, elimination theories designed to explain the cellular basis of natural self-tolerance (*2, 3*) should be revised. Investigation of systems of autosensitization, in addition to elucidating problems of theoretical interest, may suggest new ways of understanding and modifying the course of human autoimmune disease (*1*).

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### References and Notes

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