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Cell-mediated Autoimmunity: Antigen Reactive Lymphocytes recruit Specific Effector Lymphocytes

AN individual's immune system is naturally tolerant of his own body's components. Autoimmune diseases are caused by a loss of this self-tolerance and an attack against self-antigens perpetrated by the immune system. The immune damage in such diseases is often mediated directly by auto-sensitized T lymphocytes rather than by antibodies[1]. The cellular basis of T lymphocyte self-tolerance has been studied in our laboratory using *in vitro* methods of autosensitization, and we found that potentially self-reactive lymphocytes exist in healthy rats and mice[2,3]. These lymphocytes bear surface receptors which can specifically recognize self-antigens[4]. Tolerance to these antigens, however, appears to be maintained in the intact rat by the action of factors present in normal serum[5]. The factors function specifically to prevent recognition of self-antigens and, therefore, block immune activation of the potentially self-reactive lymphocytes; loss or inactivation in cell culture allows self-recognition and autosensitization. The autosensitized lymphocytes can then produce immune effects *in vivo*. Injection of such lymphocytes into the foot pads of syngeneic rats leads to enlargement of the regional popliteal lymph nodes and to the development within these nodes of effector lymphocytes which specifically damage syngeneic but not foreign target cells in culture[6].

In this system, recognition of self-antigens and the induction of autosensitization can take place only *in vitro* in the absence of the inhibitory serum factors. Hence, the development of specific effector lymphocytes in the lymph node is triggered by the autosensitization induced *in vitro*, and not by the presence of self-antigens. This system thus makes it possible to study the evolution of effector lymphocytes as distinct from the process of antigen-recognition.

Here I report the results of an experiment to identify the origin of the specific effector lymphocytes which appear following autosensitization. Two likely alternatives were considered: (1) the effector lymphocytes are the clonal descendants of the T lymphocytes which were initially autosensitized *in vitro*, or (2) the original autosensitized lymphocytes do not differentiate into effector lymphocytes but recruit effector lymphocytes from within the lymph nodes. Either the autosensitized

lymphocytes or the recipient rats were irradiated before the lymphocytes were injected, so that proliferation and differentiation of the lymphocytes derived from either source could be inhibited.

Autosensitization was induced by incubating (37° C, 10% CO_2 in moist air) 28×10^6 lymphoctyes in 4 ml. of Dulbecco's modification of Eagle's medium (EM), together with each monolayer of 2×10^6 Lewis fibroblasts in 60 mm plastic Petri dishes for 18 h as described[6]. No serum was added to the culture medium. The lymphocytes were washed from the monolayer, suspended in phosphate buffered saline, *p*H 7.2 (PBS), and 10^7 lymphocytes in 0.2 ml. PBS were injected. Irradiation of the sensitized lymphocytes (1,000 R) or the recipient rats (800 R) was done with a cobalt-60 gamma source. Table 1 shows the response of syngeneic popliteal lymph nodes measured 6 days after the injection of the thymus lymphocytes.

Normal Lewis lymph nodes were found to contain 1.7×10^6 cells and the lysis produced by the cells against Lewis target fibroblasts was about 12%. The injection of unsensitized Lewis thymus lymphocytes into normal rats did not affect these values. The lymph nodes of irradiated rats had about half of the normal number of cells (0.9×10^6) despite the injec-

Table 1 Syngeneic Lymph Node Response to Autosensitized Thymus Lymphocytes; Effects of Irradiating the Sensitized Lymphocytes or the Recipient Rats

Lewis lymphocytes injected	Lewis recipient rats	No. of cells per lymph node ($\times 10^6$)	% Lysis of Lewis target fibroblasts*
None	Normal	1.7	12.2±4.1
Unsensitized	Normal	1.8	10.4±2.3
	Irradiated	0.9	9.2±1.7
Autosensitized	Normal	28.3	71.4±2.0
	Irradiated	4.2	13.3±2.0
Autosensitized and irradiated	Normal	28.3	75.5±3.8
	Irradiated	4.3	14.9±2.7

* Suspensions of lymph node cells containing 6×10^6 cells in 1.5 ml. EM+15% horse serum were incubated in triplicate for 65 h with target monolayers of 0.5×10^6 fibroblasts in 35 mm plastic Petri dishes. The target monolayers were labelled with ^{51}Cr as described[2]. Per cent lysis was computed as:

$$\frac{\%\text{ mean }^{51}\text{Cr released in test groups} - \%\text{ mean }^{51}\text{Cr released spontaneously}}{\%\text{ mean }^{51}\text{Cr released in cultures completely lysed by freezing and thawing}}$$

tion of 10^7 unsensitized lymphocytes. The lymph nodes of normal rats responded to the injection of autosensitized thymus lymphocytes by a marked increase in the number of cells (28×10^6) and the development of effector lymphocytes which lysed (71%) syngeneic target cells. Irradiation of the autosensitized lymphocytes did not prevent them from triggering this response. In contrast, irradiation of the recipient rats inhibited the increase in lymph node cell number, and suppressed the development of effector lymphocytes. It has been shown that donor lymphocytes can proliferate and differentiate in irradiated animals[7]. Hence, these findings indicate that effector lymphocytes are derived from cells within the recipient rats, and are not necessarily the clonal descendants of the original autosensitized thymus cells. Effector lymphocytes in cell-mediated autoimmunity, therefore, may be recruited by autosensitized lymphocytes.

The basic mechanisms underlying cooperation between two classes of lymphocytes are unknown. Cell cooperation, however, was found in other immune reactions such as the induction of antibody production to thymus dependent antigens[8], the cell-mediated graft-versus-host response of parental lymphocytes against F_1 hybrid mice[9], or the *in vitro* cell-mediated heterograft reaction[10]. Thus, cooperation between different classes of lymphocytes may be a general characteristic of immune responses. Preliminary studies (I. R. Cohen, in preparation) suggest that the effector lymphocytes in this system are derived from the thymus (T-cells), and that a similar process of lymphocyte recruitment can occur following sensitization to alloantigens. Thus, both autoimmune and transplantation reactions may involve cooperation between antigen-reactive and effector classes of T lymphocytes.

The mechanisms underlying this form of cell cooperation and their possible function in the regulation of cell-mediated immune reactions are among the important questions raised by these findings.

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[1] Roitt, I. M., and Doniach, D., *Brit. Med. Bull.*, **23**, 66 (1967).
[2] Cohen, I. R., Globerson, A., and Feldman, M., *J. Exp. Med.*, **133**, 834 (1971).
[3] Cohen, I. R., and Wekerle, H., *Science*, **176**, 1324 (1972).
[4] Cohen, I. R., and Wekerle, H., in *Proceedings of the Fourth International Conference on Lymphatic Tissues and Germinal Centers in Immune Reactions* (edit. by Jankovic, B. D.) (Plenum Press, New York, in the press).
[5] Wekerle, H., Cohen, I. R., and Feldman, M., *Nature New Biology*, **241**, 25 (1973).
[6] Cohen, I. R., and Wekerle, H., *J. Exp. Med.* (in the press).
[7] Sprent, J., and Miller, J. F. A. P., *Nature*, **234**, 195 (1971).
[8] *Transplantation Rev.*, **1**, 1 (1969).
[9] Cantor, H., and Asofsky, R., *J. Exp. Med.*, **135**, 764 (1972).
[10] Lonai, P., and Feldman, M., *Transplantation*, **10**, 372 (1970).

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