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Effector T lymphocyte line cells migrate to the thymus and persist there

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The thymus gland has been known for some time to be the central organ of differentiation of T lymphocytes[1,2]. Stem cells migrate into the thymus from the bone marrow, differentiate and, as competent T lymphocytes, disperse from the thymus to the periphery, where contact with specific antigen induces immune reactivity[2]. The traffic of T lymphocytes between the thymus and periphery has been thought to be unidirectional and, unless the cells are leukaemic[3] or the thymus gland has been irradiated[4], re-entry of peripheral T lymphocytes has not been detected[3]. We now report that cells from lines of functionally active T lymphocytes reactive to self or foreign antigens can migrate back into the normal thymus gland and persist there for relatively long periods in a quiescent state until activated by contact with antigen. Hence, in addition to being the seat of T lymphocyte differentiation, the thymus is open to two-way traffic with the periphery and may function as a repository of immunological memory.

The experiments described here were prompted by our investigation of the behaviour of cells of functional T lymphocyte lines. We have found that lines of rat T lymphocytes specific for the basic protein of myelin could cause experimental autoimmune encephalomyelitis (EAE) upon intravenous inoculation into naive recipient rats[5,6]. Moreover, cells from such lines, attenuated by treatment with mitomycin C or irradiation, could be used for vaccinating rats against the induction of active EAE by subsequent immunization of the rats to myelin basic protein[7,8]. To study the migrations of such cells, we labelled them with radioactive chromium-51, injected them into rats intravenously and measured the accumulation of radioactivity as an indication of the accumulation of the injected cells in various organs. We found that anti-myelin basic protein cells accumulated in the brain and spinal cord about one day before the onset of EAE (Fig. 1). Cells from lines reactive to other antigens, such as tuberculin (PPD), did not accumulate in the central nervous system. However, Fig. 1 shows that about 1% of injected anti-myelin basic protein or anti-PPD line cells accumulated in the thymus glands of recipient rats beginning 3 days after intravenous inoculation. Accumulation in the thymus was detectable only if the cells were intact and had been activated by incubation with specific antigen before inoculation. Non-activated cells did not enter the thymus, nor did activated and irradiated cells. It is noteworthy that nonactivated, or activated and irradiated cells also did not mediate EAE[7] or transfer delayed hypersensitivity (manuscript in preparation). Thus, migration to the thymus appeared to be correlated with the state of the T lymphocytes and their capacity to mediate specific immunological effects.

Experiments were then designed to answer three questions: (1) do the returned T lymphocytes persist in the thymus, (2) are they capable of responding to specific antigen and (3) do they include potential effector cells? We injected female anti-myelin basic protein or anti-PPD line cells into syngeneic male recipient rats, and two months later we assayed the proliferative responses of their thymus cells to myelin basic protein or PPD.

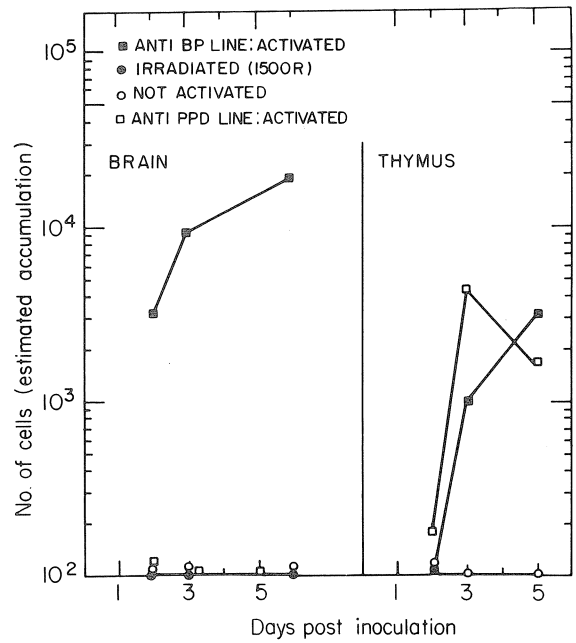

Fig. 1 Activated anti-myelin basic protein cells accumulate in the brain and thymus; activated anti-PPD line cells accumulate in the thymus. Lines of T lymphocytes specifically responsive to myelin basic protein or to PPD were raised from lymph node cells of 3-month old female Fischer rats that had been immunized with myelin basic protein-CFA[5,6]. The cells were maintained in medium containing T cell growth factor[9] and some of the cells were activated by incubation with their respective antigen, myelin basic protein or PPD in the presence of irradiated (1,500 rad) normal thymus accessory cells for 72 h (refs. 5, 6): some of these activated cells were irradiated with 1,500 rad. The line cells were labelled[10] by incubating 10^7 cells per ml Eagle's medium containing ^{51}Cr as sodium chromate (Amersham). The cells were washed by centrifugation in medium. Groups of 6 male Fischer rats were then inoculated into the tail vein with 3×10^6 labelled cells from lines of each type. On days 2, 3, 5 or 6, two rats of each group were killed and the accumulated line cells in the brains and thymuses were estimated by the c.p.m. emanating from each whole organ measured in a gamma counter. Differences between the organs of the two rats assayed at each time point were negligible. The number of cells was computed as the c.p.m. divided by the c.p.m. per cell in the inoculum. Similar results were obtained in four consecutive experiments.

The thymus glands used in the experiments were dissected free of the lymph nodes imbedded in their postero-lateral portions. The results are shown in Table 1. Control thymus cells from naive rats showed no response to myelin basic protein and only a weak response to PPD (SI = 2). The thymus cells of rats that had been inoculated intravenously with activated anti-myelin basic protein line cells and had recovered from EAE[8,11] responded weakly to myelin basic protein (SI = 3). The slight response to PPD of these thymus cells was about the same as that of naive control rats (SI = 2). Injection of anti-myelin basic protein cells did not produce a response to PPD. In contrast, thymus cells from rats that had been inoculated with activated anti-PPD cells showed a response to PPD (SI = 6) but not to myelin basic protein. Nonactivated anti-PPD cells produced no response.

To determine the immunospecificity of the cells responding to myelin basic protein and to determine their origin, they were grown in medium containing T-cell growth factor (IL-2)[9] with alternating periods of stimulation with myelin basic protein until they developed into a stable line of T lymphocytes[6]. Accessory cells added during stimulation with myelin basic protein were of male origin, as were the recipient rats from whom the thymuses were taken for culture. Thus, only the original line cells inoculated over 2 months earlier were of female origin.

Table 1 Activated female anti-myelin basic protein T lymphocyte line cells persist in male thymus and can be recovered

Female line cells inoculated into male rats		Thymus cells		Recovered anti-myelin basic protein cells				
		Proliferation Δ c.p.m. (SI)		Proliferation Δ c.p.m. (SI)				
Specificity	Treatment	Myelin basic protein	PPD	Myelin basic protein	PPD	Incidence of EAE	Karyotype Male	Female
None	None	95 (1)	921 (2)					
Anti-myelin basic protein	Activated	3,156 (3)	1,376 (2)	82,051 (13)	0 (1)	5/5	0/20	20/20
	Irradiated	0 (1)	880 (1)					
	Nonactivated	0 (1)	995 (2)					
Anti-PPD	Activated	0 (1)	4,984 (6)					
	Nonactivated	0 (1)	950 (2)					

Proliferative responses to myelin basic protein or to PPD of thymus cells from groups of five male 3-month old Fischer rats were measured[5,8]. Some of the groups had been inoculated i.v. 2 months earlier with 3×10^6 female anti-myelin basic protein or anti-PPD line cells[5,6]. Cells from the lines were either nonactivated, activated or activated and irradiated (see Fig. 1 legend). Rats that had been inoculated with anti-myelin basic protein line cells developed EAE 4 days later and recovered spontaneously[6,11]. An anti-myelin basic protein line was recovered from pooled thymuses free of lymphnodes of the male rats that had been inoculated with the female activated anti-myelin basic protein line cells[6]. The proliferative responses of these line cells to myelin basic protein and to PPD were tested[6], as was their ability to mediate EAE in naive recipient rats. The karyotype of the anti-myelin basic protein line cells was studied in 20 cells[12].

Table 1 shows that cells recovered from anti-myelin basic protein lines responded strongly and specifically to the antigen *in vitro* (SI = 13), and mediated EAE upon intravenous inoculation into naive Fischer rats. Furthermore, analysis of the chromosomes of the recovered anti-myelin basic protein cells showed that they all had the female karyotype of the original line. Therefore, the antigen-specific cells detectable in the thymus 2 months after inoculation and spontaneous recovery from EAE were cells of the donor EAE effector line. Similar results were obtained in experiments using rats and line cells of the Lewis rat strain.

These findings indicate that competent, specifically sensitized T lymphocytes can re-enter the thymus and persist there. Thus, the thymus is connected to the periphery by a two-way flow of potential effector lymphocytes. The T lymphocytes that leave the thymus appear to be those induced to differentiate from precursors by contact with epithelial cells of the thymic stroma[13] or with macrophage-like cells[14]. This stage of differentiation is not dependent on clonal recognition of specific antigens but may involve the acquisition of the restriction of antigen recognition to association with particular products of the major histocompatibility complex[15,16]. In contrast, the T lymphocytes that return to the thymus from the periphery appear to be induced to do so in response to antigen. The cell lines used in these experiments were developed from cells of antigen-primed rats by their proliferative responses to specific antigens and by their ability to respond to T cell growth factor[5,6]. Furthermore, the cells did not return to the thymus and persist there unless they had had contact with their specific antigen just before inoculation. Thus, the capacity of anti-myelin basic protein T lymphocytes to return to the thymus, to persist there and to mediate EAE appeared to be part of the programme of differentiation induced by the antigen. In their persistence and response to specific recall, these cells are a token of immunological memory.

We also have been able to recover EAE effector T lymphocytes from the thymuses of rats that had recovered from EAE induced by active immunization to BP in adjuvant[11]. Thus, the thymic immigration of effector T lymphocytes would seem to include cells generated endogenously as well as exogenous line cells. The anatomical site of residence in the thymus of the returned T lymphocytes is unknown, but it is probable that they comprise part of the pool of relatively mature longlived cells residing in the medulla[17].

The present observation raises a number of questions. Are all populations of sensitized lymphocytes represented in the thymus? Are some sensitized T lymphocytes specifically destined to return to the thymus because they bear particular receptors, or is the return a random process in which all sets of T lymphocytes participate equally? Does the persistence in the thymus of these returned cells have a role in immunological memory? Why and how are persistent autoimmune effector T lymphocytes kept quiescent? Is activation of such cells a factor in the progression or exacerbation of autoimmune diseases? Or, on the contrary, does the persistence of autoimmune cells help maintain self-tolerance by priming anti-idiotypic networks[18]?

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