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Vaccination against autoimmune encephalomyelitis with T-lymphocite line cells reactive against myelin basic protein

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Despite differences in initiating events and pathophysiology, the aetiological agents of all autoimmune diseases are lymphocytes specifically reactive against normal constituents of the individual. Recently we have isolated and grown as a cell line rat T lymphocytes reactive against myelin basic protein (BP)[1]. This T-cell line originated from rats in which we had induced experimental autoimmune encephalomyelitis (EAE) by immunizing them against BP. Inoculation of syngeneic rats with the T-cell line led to the relatively rapid onset of EAE[1]. We report here that attenuation of this cell line provides an agent for establishing resistance to induction of active EAE. Intravenous (i.v.) inoculation of syngeneic rats with cells of the line attenuated by treatment with irradiation or mitomycin C augmented resistance to EAE caused by an encephalitogenic challenge with BP. Thus, aetiological agents of autoimmune disease, like those of microbial disease, when suitably attenuated can be used as effective vaccines.

EAE can be induced in susceptible animals such as rats, guinea pigs, rabbits, monkeys[2] or man[3] by injecting them with BP emulsified in an adjuvant such as complete Freund's adjuvant (CFA). In Lewis rats the disease is characterized by paralysis that is most marked in the tail and hind limbs and which starts usually ~12 days after a single injection of BP in CFA. Histologically the central nervous system shows perivascular infiltrates of mononuclear cells[4]. Unless the rats are aged or have undergone splenectomy or thymectomy[5] they recover spontaneously from clinical paralysis after a number of days. To study the pathophysiology of EAE we have isolated and propagated *in vitro* a line of Lewis rat T lymphocytes that reacts only against BP, designated Z1a (ref. 1). We found that i.v. inoculation of as few as 10^5 cells of the Z1a line led to the onset of paralysis in ~4 days. Inoculation of 10^6 or more cells produced paralysis in ~2–3 days. Most rats recovered from this form of EAE if properly nursed during their paralysis. Table 1 shows the specificity of the proliferative response of the anti-BP Z1a line compared with that of the Z1c line which had been selected for its reactivity against another antigen, the purified protein derivative (PPD) of the mycobacteria present in CFA. The cells of each line responded to its specific antigen, and were also activated by the T-cell mitogen concanavalin A (Con A). Essentially all the cells in both the Z1a and Z1c lines proved to be positive for a T-cell marker using a specific monoclonal antibody (Sera-lab, UK; clone W3/13 HLK) in an immunofluorescence assay[6].

We investigated the effect of attenuating the Z1a line by inhibiting its cell division. Table 2 shows that i.v. injection of 1×10^7 untreated cells of the Z1a line into syngeneic Lewis rats produced EAE in 18 of 20 rats within 2–3 days. Irradiation of the cells with 1,500 rad or treatment with mitomycin C, agents that block cell division, abrogated the ability of these cells to cause EAE. None of 25 rats that received Z1a cells treated in this way developed EAE. Furthermore, inoculation of untreated cells of the Z1c line also failed to induce EAE. Thus, induction of EAE is a function of the specific anti-BP Z1a line, a property lost after irradiation or treatment with mitomycin C.

We then tested whether inoculation with cells incapable of inducing EAE could affect the susceptibility of rats to active induction of EAE by later challenge with BP in CFA. Table 3 shows that untreated Lewis rats were highly susceptible to induction of EAE on injection with BP in CFA; 69 of 71 rats developed disease. Intravenous inoculation of cells of the Z1c anti-PPD line, either untreated or irradiated, did not affect this susceptibility and EAE was induced in all 20 rats challenged with BP in CFA. In contrast, a single i.v. injection of 1×10^7 Z1a cells attenuated by treatment with mitomycin C or irradiation led to significantly increased resistance to induction of EAE. Only 14 of a total of 40 rats showed any signs of paralysis and the degree of the paralysis in these rats was judged to be much milder than that appearing in the other groups. Thus it seems that vaccination with attenuated autoimmune T lymphocytes

Table 1 Anti-BP and anti-PPD T-cell lines are immunospecific

T-cell line	Proliferative response (c.p.m. $\times 10^{-3} \pm$ s.d.) No antigen	BP	PPD	Con A
Anti-BP (Z1a)	1.7 ± 0.3	48.7 ± 6.1	1.9 ± 0.4	71.4 ± 9.2
Anti-PPD (Z1c)	1.4 ± 0.7	1.2 ± 0.4	77.9 ± 10.4	82.3 ± 12.7

The Z1a and Z1c cell lines originated from the same draining lymph node cell population obtained from female Lewis rats immunized with BP in CFA as described elsewhere[1]. To develop the cell lines, Lewis rats were injected in each footpad with 0.05 ml containing BP (25 μg, extracted from guinea pig spinal cords[10] emulsified in equal volumes of phosphate-buffered saline and CFA containing 4 mg ml^{-1} of *Mycobacterium tuberculosis* $H_{37}Ra$ (Difco). On day 9, the draining lymph nodes were removed and a single-cell suspension prepared. The cells were then selected *in vitro* for BP or PPD by culturing them with either antigen for 72 h. The lymphoblasts that were generated were separated by a discontinuous Ficoll gradient and propagated and maintained *in vitro* as a cell line for several months in medium enriched with T-cell growth factor as reported elsewhere[1]. The proliferative responses of the T-cell lines were tested *in vitro* as follows[1]. Briefly, 2.5×10^4 cells of either Z1a or Z1c cells were cultured in quadruplicates in flat-bottom microtitre wells in 0.2 ml of Eagle's medium supplemented with 1% fresh autologous rat serum, 2-mercaptoethanol (5×10^{-5} M), L-glutamine (2×10^{-3} M) and antibiotics (streptomycin and penicillin) with added irradiated (1,500rad) normal syngeneic lymph node cells as accessory cells (5×10^6 cells ml^{-1}) and antigens, BP (50 μg ml^{-1}), or PPD (25 μg ml^{-1}; Staten Serum Institut) or Con A (2.5 μg ml^{-1}; Miles-Yeda, Israel). After 24 h the cultures were pulsed with ^{3}H-thymidine (1 μCi per well, specific activity 10 Ci mmol^{-1}; Nuclear Research Centre, Israel) for 16 h. The cells were then collected on glass fibres using an automatic collector and thymidine incorporation measured in a liquid scintillation counter.

Table 2 Attenuated T lymphocytes of the Z1a anti-BP line do not produce EAE

Line	Inoculation of T-cell lines Treatment	Incidence of EAE
Anti-BP (Z1a)	Untreated	18/20
	Irradiated	0/15
	Mitomycin C	0/10
Anti-PPD (Z1c)	Untreated	0/20

Healthy female Lewis rats (2–3 months old) were injected i.v. with 1×10^7 cells of T-lymphoblast cell lines specifically reactive against BP (Z1a) or PPD (Z1c). Before inoculating the cell lines into normal syngeneic animals, they were re-stimulated *in vitro* with the relevant antigen, in the presence of irradiated (1,500 rad) syngeneic accessory cells for 72 h (ref. 1). The cells, >80% lymphoblasts, were then collected and injected, either untreated or attenuated by irradiation (1,500 rad) from a ^{60}Co source, or treatment with mitomycin C (50 μg per 10^7 cells per ml; Sigma) at 37°C for 40 min. The treated cells were washed extensively before being inoculated. EAE was diagnosed clinically by overt paralysis of the hind limbs and histologically by perivascular mononuclear cell infiltration of the central nervous system[4].

Table 3 Attenuated anti-BP T-cell line vaccinates rats against induction of EAE

Vaccination with T-cell line	Incidence of EAE in response to injection of BP in CFA	% Inhibition of EAE
None	69/71	—
Anti-PPD (Z1c)		
Untreated	10/10	0
Irradiated	10/10	0
Anti-BP (Z1a)		
Irradiated	8/25*	68
Mitomycin C	6/15*	60

EAE was induced in naive Lewis rats (2–3 months old) or in animals that had been vaccinated i.v. 3 weeks earlier with 10^7 cells of the anti-PPD Z1c T-cell line or with cells of the anti-BP Z1a T-cell line that were either irradiated (1,500 rad) or treated with mitomycin C, as described in Table 2 legend. EAE was induced by injecting BP in CFA into the hind footpads of the animals, as described in Table 1 legend.
*$P < 0.001$.

provided protection against active EAE for about 65% of the rats.

We do not know the mechanism by which the attenuated T lymphocytes increased resistance to induction of EAE; however, it seems reasonable to suspect that some process of immunity was involved. The Z1a anti-BP lymphocytes probably differed from the ineffective Z1c anti-PPD lymphocytes in the structure of their antigen receptors (Table 1). Antigen receptors of T lymphocytes[7] as well as of B lymphocytes or antibodies[8] can be immunogenic. Immunity against antigen receptors, anti-idiotypic immunity, has been proposed to serve as a mechanism that regulates immune responses by suppressing or activating specific clones of lymphocytes bearing the target receptors[9]. Vaccination with attenuated Z1a cells might have produced an immune response against endogenous clones of lymphocytes with anti-BP receptors. As anti-BP clones are the aetiological agents of EAE, development of the disease would be inhibited. Therefore, our results could be explained by anti-receptor immunity raised against the autoimmune lymphocytes that mediate EAE. However, other explanations are possible and the anti-receptor hypothesis must be tested experimentally.

Whatever the mechanism of protection, the procedure described here can be conceptually related to vaccination against infectious diseases in which inoculation of an attenuated agent of disease induces a degree of protection against the virulent pathogen. In the case of autoimmunity, the aetiological agent of disease is not a microbe, but arises within the immune system of the individual. Our results indicate that an artificially induced autoimmune disease may be mitigated or prevented by vaccination against specific effector lymphocytes. A different but related problem is posed by the need to treat the spontaneous, often chronic processes that characterize the important autoimmune diseases of man.

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