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Anti-Idiotypic Network Induced by T Cell Vaccination Against Experimental Autoimmune Encephalomyelitis

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In a study of the mechanism of resistance to autoimmune disease induced by T cell vaccination, rats were vaccinated against experimental autoimmune encephalomyelitis (EAE) by injecting them once in the hind footpads with a subencephalitogenic dose (10^4) of a clone of T lymphocytes specific for myelin basic protein (BP). The response to vaccination was assayed by challenging the rats with an encephalitogenic dose (3×10^6) of T lymphocytes of this BP-specific clone. Five to six days after vaccination, the cells responsible for mediating resistance to adoptively transferred EAE were concentrated in the popliteal lymph nodes draining the vaccination site. Transfer of the draining lymph node cells to unvaccinated rats led to loss of resistance in the donor rats and acquisition of resistance by the recipient rats. Limiting-dilution cultures of the draining lymph node cells were established with irradiated cells of the BP-specific clone as stimulators. Two sets of T lymphocytes specifically responsive to the BP-specific T cells from the clone were isolated: $CD4^+CD8^-$ helper and $CD4^-CD8^+$ suppressor cells. The helper T cells, like the BP antigen, specifically stimulated the BP-specific vaccinating clone. In contrast, the suppressor T cells specifically suppressed the response of the BP-specific vaccinating clone to its BP antigen. These results suggest that T cell vaccination induces resistance to autoimmune disease by activating an anti-idiotypic network.

WE HAVE INDUCED RESISTANCE to various experimental autoimmune diseases by vaccinating rats or mice with autoimmune T lymphocytes of the antigen specificity relevant to the particular disease (*1–4*). Our strategy for studying and manipulating autoimmune disease experimentally has been to isolate, in culture, lines and clones of T lymphocytes functionally active in producing experimental autoimmune encephalomyelitis (EAE), experimental autoimmune thyroiditis, or adjuvant arthritis (*4–7*). These T cells have been useful in investigating factors in pathogenesis such as the identity of critical self-epitopes (*8, 9*), traffic of autoimmune T lymphocytes through blood vessels to target organs (*10, 11*), and interactions between autoimmune T lymphocytes and target tissues (*12, 13*). Autoimmune T lymphocytes, in addition to probing pathogenesis, can be rendered avirulent and used to induce resistance to the specific autoimmune disease with which they are associated. We have called this practice T cell vaccination by its analogy to the use of attenuated agents of infectious disease for similar ends (*2*).

At first, we used irradiated autoimmune effector T cells for vaccination (*2*). Recently we found vaccination to be more effective when we used T cells treated by hydrostatic pressure or chemical cross-linkers (*14, 15*). However, some untreated autoimmune effector clones can also be used as vaccine if a dose of cells below the threshold number needed for disease is administered (*4, 16*). Common to all forms of vaccination is the requirement that the T cells be activated, prior to inoculation, by incubation with specific antigen or a T cell mitogen such as concanavalin A together with antigen-presenting cells (*10*). T cell vaccination induced lasting remission of established autoimmune disease and prevented the development of subsequent induction of disease (*15*).

The strategy in the present study was to vaccinate rats in the hind footpads with cells of a T cell clone specifically directed against myelin basic protein (anti-BP T cells) and to assay the draining popliteal lymph node (PLN) and a distal lymph node for the presence of cells able to recognize the anti-BP T cells and to mediate resistance to adoptive EAE. We used the anti-BP T cells designated Z1a, which was isolated in 1980 (*17*). Recent analysis of the T cell receptor gene rearrangements of Z1a indicates that it is a single clone (*18*). Clone Z1a is reactive to an epitope within the encephalitogenic peptide (amino acids 68–88) of myelin basic protein (*9*). Z1a regularly produces EAE when injected intravenously at doses of 10^5 cells or greater, but induces resistance to EAE without first causing either clinical EAE or histologic lesions of EAE when injected at doses of 10^3 to 10^4 cells (*16*).

Table 1 shows the results of an experiment in which Lewis rats were vaccinated in the hind footpads with 10^4 mitogen-activated Z1a T cells. At various times the draining PLN and distal cervical lymph nodes (CLN) were assayed for T cell proliferative responses to the Z1a clone or to control clone A2b. [A2b is a helper T cell clone that responds to *Mycobacterium tuberculosis* and to joint cartilage (*8*) and can be used as a potent vaccine against adjuvant arthritis, but not against EAE (*15*).] Before vaccination and 2 days after vaccination, the rats showed background proliferative responses in PLN and CLN populations to both Z1a and A2b clones. However, on day 5 after vaccination (and also on day 6 in other experiments), the PLN, but not the CLN, showed a specific response to vaccinating clone Z1a. By day 11 (and also on day 9 in other experiments), the response had become systemic and both PLN and CLN populations responded to Z1a specifically.

To learn whether the response to Z1a was associated with protection against EAE, we transferred the responding PLN cells from vaccinated to naïve rats. In the experiment shown in Table 2, Lewis rats were vaccinated in the hind footpads and 6 days later the draining PLN of some rats were excised. The PLN cells were activated by a mitogen and transferred intraperitoneally into naïve recipient rats. Control rats either were not vaccinated or were inoculated with the mitogen-activated PLN cells of unvaccinated rats. One day later all the rats (unvaccinated controls, PLN donors, and PLN recipients) were tested for susceptibility to EAE produced by intravenous inoculation of 3×10^6 Z1a cells. All of the 17 control rats (unvaccinated or recipients of PLN of unvaccinated rats) developed EAE, fatal in 14 of 17 animals (the sum of the numbers of animals in groups 1, 2, and 5). In contrast, none of the 15 vaccinated rats with intact PLN developed EAE (group 3). Thus vaccination with 10^4 Z1a cells was markedly effective ($P < 0.02$, with Bonferroni correction).

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However, on day 6, the cells responsible for vaccination were concentrated in the PLN. Excision of the PLN robbed the vaccinated rats of their resistance (group 4). In contrast, the naïve recipients of these PLN cells (group 6) acquired a significant measure of resistance [incidence of EAE was 3 of 9 compared to 17 of 17, the total number of animals in control groups 1, 2, and 5 ($P < 0.02$); mortality was 1 of 9 compared to 14 in 17 ($P < 0.02$)]. After day 6, resistance became systemic and could be transferred to recipient rats without costing the donors their protection. Thus, the T cell response to Z1a, confined to the draining PLN on days 5 and 6, marked the mechanism of resistance; resistance to EAE depended in large measure on retaining or receiving these PLN cells.

To identify the cells producing the response to Z1a in the PLN of the vaccinated rats, we set up limiting-dilution cultures of PLN cells, with irradiated Z1a cells as stimulators. The responding cultures that initially contained the fewest vaccinated PLN cells were subjected to repeated courses of stimulation with Z1a to derive T cell lines directed against Z1a (anti-Z1a T cells). Of more than 30 responding cultures, 7 have yielded enough cells to enable us to determine both their phenotype and their functions in vitro. Four of the seven anti-Z1a cultures were found by fluorescence flow cytometry to bear the marker of suppressor or cytotoxic T cells; the cells were positive for OX8 ($CD8^+$) and negative for W3/25 ($CD4^-$). The other three cultures were composed of helper T cells positive for W3/25 ($CD4^+$) and negative for OX8 ($CD8^-$).

Table 3 illustrates the effects of the $CD4^+$ and $CD8^+$ anti-Z1a T cells on the proliferation of Z1a. The anti-Z1a T cells were irradiated to prevent their own proliferation from interfering with the helper and suppressor assays. Two of the three $CD4^+$ T helper cells (TR1 and TR4) strongly activated Z1a in the absence of BP antigen, and they augmented somewhat the response of Z1a in the presence of BP. In contrast, the four $CD8^+$ cells suppressed the response of Z1a to BP by 51% to 83%.

Both the stimulatory and the suppressive effects were specific for the Z1a clone (Table 4). Although the number of anti-Z1a cells in each well was only 10^4 compared to 10^5 in the experiment shown in Table 3, $CD4^+$ T cell TR1 caused a greater than threefold stimulation of Z1a in the absence of antigen. TR1 induced no signficant stimulation of control clone A2b reactive to *M. tuberculosis*. The other $CD4^+$ cell, TR2, failed to stimulate. In contrast, the two $CD8^+$ cells, TR7 and TR12, suppressed by more than 50% the response of Z1a to BP, but not the response of A2b to its antigen *M. tuberculosis*. In other experiments, we found that clone Z1a and the $CD4^+$ anti-Z1a clone TR1 were mutually stimulatory; irradiated Z1a cells stimulated TR1 and irradiated TR1 stimulated Z1a specifically. As expected, clone Z1a but not TR1 responded to the BP antigen that induces EAE.

Immune responses to clonotypic markers of lymphocytes may be termed anti-idiotypic responses (*19*). Vaccination with helper clone Z1a induced T cells that recognized clone Z1a but not A2b, a syngeneic helper clone with a different self-antigen specificity, and vice versa (*15*). The anti-Z1a T cells also failed to react to syngeneic helper T cells responsive to other unrelated antigens such as ovalbumin. Thus, we may term the anti-vaccine response an anti-idiotypic response (*19*). It is likely that the idiotype of clone Z1a, similar to other idiotypes, is created by its antigen receptor (*19*), in this case the receptor for the BP autoantigen. Thus T cell vaccination appears to induce an anti-idio-

Table 1. Specific anti-Z1a PLN proliferative response induced by T cell vaccination with Z1a. Groups of five Lewis rats were vaccinated in the hind footpads by injection of 10^4 activated clone Z1a cells. Clone Z1a was isolated and maintained as described (*17*). The Z1a cells (0.3×10^6 cells per milliliter) were activated by incubation for 48 hours with the T cell mitogen concanavalin A (1.5 μg/ml; Bio-Yeda, Israel), and irradiated syngeneic thymocytes (2500 rads) as accessory cells (10^7 cells per milliliter). At 2, 5, and 11 days later, PLN cells or CLN cells were assayed for their proliferative responses to irradiated (2500 rads) stimulator cells of clone Z1a or control clone A2b. Both clones Z1a and A2b (*23*) were derived from Lewis rats and had the phenotypes of T helper cells. The lymph node cells (2×10^5) were incubated in triplicate with the activated, irradiated stimulator clones (10^5) in U-shaped 96-well microtiter plates (Greiner, West Germany) in 0.2 of Dulbecco's modified Eagle's medium (DMEM) containing 1 m*M* glutamine (Bio-Lab, Jerusalem, Israel), 2-mercaptoethanol (5×10^{-5} *M*), antibiotics, and 1% fresh autologous rat serum. After 3 days of culture, each well was pulsed with 1 μCi of [^{3}H]thymidine (Nuclear Research, Negev, Israel) for 18 hours. The cultures were then harvested on fiberglass filters and the incorporation of thymidine was measured with a liquid scintillation counter. Results are recorded as counts per minute $\times 10^{-3}$ in test wells minus counts per minute $\times 10^{-3}$ in control wells without stimulator clones Z1a or A2b (approximately 2000 cpm).

Days after vaccination	Source of cells	Proliferative response to clones	
		Z1a	A2B
	Unvaccinated controls		
	PLN	2 ± 0.2	2 ± 1
	CLN	2 ± 0.1	3 ± 1
	Vaccinated with Z1a		
2	PLN	4 ± 1	1 ± 0.1
	CLN	4 ± 2	3 ± 0.3
5	PLN	23 ± 3	4 ± 0.6
	CLN	6 ± 1	2 ± 0.5
11	PLN	27 ± 2	5 ± 1
	CLN	20 ± 1	7 ± 2

Table 2. Footpad vaccination against adoptive EAE: resistance to EAE mediated by draining PLN cells. Lewis rats were vaccinated in the hind footpads with 10^4 activated Z1a clone cells as described in the legend to Table 1. On day 6, the rats were anesthetized with ether and the draining popliteal PLNs of some of the rats were excised. The PLN cells were activated by incubation for 48 hours with concanavalin A (1.5 μg/ml). Cells (20×10^6) were then transferred intraperitoneally to each naïve Lewis recipient rat. One day later the originally vaccinated rats, with or without excision of the PLN, and the recipients of the PLN cells were tested for susceptibility to EAE by intraperitoneal inoculation with 3×10^6 activated Z1a cells (*17, 24*). Control rats were not vaccinated and some of their PLN cells were transferred to recipient rats which were challenged with Z1a as above. Incidence of paralysis and mortality were observed. Successful vaccination with 10^4 Z1a cells prevented the histologic lesions of adoptive EAE, as it prevented the clinical manifestations of EAE (*16*).

Group	Rats	Susceptibility to adoptive EAE induced by Z1a	
		Incidence	Mortality
	Unvaccinated controls		
1	PLN intact	7/7	5/7
2	PLN excised	5/5	5/5
	Vaccinated		
3	PLN intact	0/15	0/15
4	PLN excised (day 6)	6/6	2/6
	PLN recipients		
5	Unvaccinated PLN	5/5	4/5
6	Vaccinated PLN	3/9	1/9

typic response to the T cell vaccine that includes both $CD4^+$ and $CD8^+$ T cells. Human anti-idiotypic $CD8^+$ T cell responses specific for receptors that recognize viral antigens (*20*) or alloantigens (*21*) have been reported.

One may hypothesize that the anti-idiotypic $CD8^+$ cells contributed to EAE resistance by suppressing the function of the T cells in vivo as they did their proliferation in vitro. It is possible that the anti-idiotypic $CD4^+$ T cells, although stimulating in vitro, might in vivo aid resistance by inducing delayed-type hypersensitivity to the autoimmune effector cells or by driving the idiotype–anti-idiotype network to enhance suppression (*19*). Administration in vivo of individual clones of anti-idiotypic T cells might shed light on these points.

Resistance to EAE produced by 3×10^6 Z1a cells was effected by a single inoculation of 10^4 Z1a cells. Vaccination with 10^4 Z1a cells also produced significant resistance to EAE induced by active immunization to BP in adjuvant (*16*). This exquisite degree of sensitivity to T cell vaccination suggests a recall of existing anti-idiotypic immunity to anti-BP T cells, rather than induction of a new, primary immune response.

Not all autoimmune T cells are able to induce an anti-idiotypic response or resistance to disease when administered at a single low dose of cells. For example, vaccination against adjuvant arthritis with clone A2b required administration of several doses of 2×10^7 cells that had been treated by hydrostatic pressure or chemical cross-linkers (*15*). Thus, there may exist variable degrees of sensitivity to different T cells or to their idiotypes, as well as differences between the disease models and their susceptibility to regulation by vaccination.

Regarding the clinical application of T cell vaccination, a recent report by Edelson *et al.* (*22*) indicates that a form of T cell vaccination can be effective in inducing regression of T cell tumors in human patients. Our results suggest that the anti-idiotypic response to the vaccinating T cells may serve to monitor patients' responses.

Table 3. Effects on Z1a cells of anti-Z1a $CD4^+$ and $CD8^+$ cells. Lewis rats were vaccinated with 10^4 activated Z1a clone cells in the hind footpads. On day 6, the draining lymph nodes were excised and suspensions of lymph node cells (2×10^5 cells) were cultured with irradiated (2000 rads) Z1a (10^5 cells) in 0.2 ml of DMEM stimulation medium as described in Table 1. After 4 days, the blast cells were collected, washed, counted and seeded at 1, 10, and 100 cells per well into 96-well microtiter plates in 0.2 ml of proliferation medium supplemented with 10% (vol/vol) supernatant from concanavalin A–stimulated splenocytes as a source of T cell growth factor, 10% heat-inactivated horse serum, 1 m*M* sodium pyruvate, and nonessential amino acids. Resulting clones were expanded in propagation medium as described (*23*). After 7 to 14 days, the clones were collected, washed, counted, and restimulated with activated irradiated Z1a cells (1:1 ratio) and irradiated (2000 rads) autologous thymocytes as accessory cells (10^5 cells per well), and then transferred back into 96-well microtiter plates in propagation medium (10^2 to 10^5 cells per well). TR1, TR2, TR4, TR7–9, and TR12 cells were propagated in this manner for 6 weeks before study. The effect of the anti-Z1a cells on the proliferation of Z1a to its antigen myelin basic protein (10 μg/ml) was examined by transferring activated, irradiated (2000 rads) anti-Z1a clones into U-shaped wells (10^5 cells per well) in triplicate containing 10^6 irradiated accessory cells and 2.5×10^4 Z1a cells with or without myelin basic protein. After 72 hours of incubation, each well was treated with [^{3}H]thymidine for 18 hours, harvested, and counted. Ascites fluid containing monoclonal antibodies (Serotec) was used to analyze membrane markers. W3/25 is a mouse antibody to rat T helper cells and OX8 is a mouse antibody to rat T suppressor and cytotoxic cells. Rhodamine-labeled rabbit antibodies to mouse immunoglobulin G (H and L chains) were obtained from Bio-Yeda, Israel. Fluorescence analysis was performed with a fluorescence-activated cell sorter (FACS) (Becton Dickinson) as described (*23, 24*). At least 10^4 viable cells were analyzed for fluorescence intensity at different gains. ND, not determined. Proliferative response is measured as the change in counts per minute and is recorded as the mean ± SD × 10^{-3}.

Irradiated anti-Z1a cells	Phenotype	Proliferative response	
		Z1a alone	Z1a + BP
None		5 ± 1	183 ± 12
TR1	$CD4^+$	110 ± 11	189 ± 15
TR2	$CD4^+$	13 ± 2	218 ± 17
TR4	$CD4^+$	76 ± 13	226 ± 19
TR7	$CD8^+$	ND	31 ± 3
TR8	$CD8^+$	ND	59 ± 7
TR9	$CD8^+$	12 ± 1	48 ± 2
TR12	$CD8^+$	4 ± 1	90 ± 10

Table 4. Immunological specificity of anti-Z1a stimulation or suppression. Activated, irradiated (2000 rads) anti-Z1a T lymphocytes were seeded into U-shaped 96-microtiter wells (10^4 cells per well) in triplicate in the presence of 10^6 irradiated thymus cells, and 2.5×10^4 Z1a or A2b clone cells in 0.2 ml of proliferation medium. After 72 hours, the cultures were incubated with [^{3}H]thymidine for 18 hours harvested, and counted. Results are presented as counts per minute ×10^{-3} (±SD).

Irradiated anti-Z1a cells	Phenotype	Proliferative response			
		Z1a alone	A2b alone	Z1a + BP	A2b + MT
None		8 ± 1	9 ± 2	99 ± 12	92 ± 11
TR1	CD4	26 ± 3	12 ± 1	93 ± 3	87 ± 4
TR2	CD4	8 ± 0.2	9 ± 0.6	99 ± 10	106 ± 9
TR7	CD8	12 ± 1	9 ± 1	54 ± 14	152 ± 18
TR12	CD8	4 ± 1	5 ± 1	45 ± 4	82 ± 10

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