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Immunology

Therapeutic vaccination against adjuvant arthritis using autoimmune T cells treated with hydrostatic pressure

(autoimmune disease/experimental autoimmune encephalomyelitis)

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ABSTRACT **An ideal treatment for autoimmune diseases would be a nontoxic means of specifically neutralizing the autoreactive lymphocytes responsible for the disease. This goal has been realized in experimental autoimmunity models by immunizing rats or mice against their own autoimmune cells such that the animals generate an immune response specifically repressive to the disease-producing lymphocytes. This maneuver, termed lymphocyte vaccination, was demonstrated to be effective using some, but not all, autoimmune helper T-lymphocyte lines. We now report that T lymphocytes, otherwise incapable of triggering an immune response, can be transformed into effective immunogens by treating the cells *in vitro* with hydrostatic pressure. Clone A2b, as effector clone that recognized cartilage proteoglycan and caused adjuvant arthritis in Lewis rats, is such a cell. Untreated A2b could not trigger an immune response, but inoculating rats with pressure-treated A2b induced early remission of established adjuvant arthritis as well as resistance to subsequent disease. Specific resistance to arthritis was associated with anti-idiotypic T-cell reactivity to clone A2b and could be transferred from vaccinated rats to naive recipients using donor lymphoid cells. Aggregation of T-lymphocyte membrane components appeared to be important for an immune response because the effects of hydrostatic pressure could be reproduced by treatment of A2b with chemical cross-linkers or with agents disrupting the cytoskeleton. Populations of lymph node cells from antigen-primed rats, when treated with hydrostatic pressure, could also induce suppression of disease. Thus, effective vaccines can be developed without having to isolate the autoimmune T lymphocytes as lines or clones. These results demonstrate that effector T lymphocytes suitably treated may serve as agents for specifically controlling the immune system.**

Organ-specific autoimmune diseases are caused by clones of autoimmune effector T lymphocytes that attack the otherwise normal tissues of the individual (1). Accordingly, it should be possible to treat autoimmune processes by inducing an immune response specifically directed against the pernicious autoimmune effector clones.

This idea was realized experimentally when we isolated lines and clones of autoimmune helper T lymphocytes capable of causing various autoimmune diseases in rats or mice (2, 3). It was observed that some of these T lymphocytes, made avirulent by irradiation, could be used to induce resistance to specific autoimmune diseases produced by virulent autoimmune T lymphocytes of similar specificity (4, 5).

The present report relates to adjuvant arthritis, a disease that is induced in susceptible strains of rats with *Mycobacterium tuberculosis* in oil, a mixture known as complete Freund's adjuvant (6). Helper T lymphocytes were raised as lines and clones by immunizing Lewis strain rats with *M. tuberculosis* and selecting *M. tuberculosis*-specific T lymphocytes *in vitro* (7). One clone, designated A2b, was found to produce arthritis in irradiated Lewis rats (8). This clone proliferated *in vitro* in response to an epitope of *M. tuberculosis*, but also reacted to the proteoglycan of joint cartilage (9). Thus, arthritogenic A2b identified a shared epitope between *M. tuberculosis* and a self-component of the joint, an epitope that explained how a foreign bacterial antigen could induce an autoimmune disease (10). Helper T-lymphocyte responses to the cross-reactive *M. tuberculosis* antigen also have been detected in the peripheral blood and synovial fluid of rheumatoid arthritis patients (11). Autoimmune clone A2b, irradiated or not, did not induce resistance to active adjuvant arthritis (8).

Our aim was to learn how clone A2b and other T lymphocytes might be converted into specific vaccines against autoimmunity. We reasoned that vaccination might be enhanced by measures that augmented the immunogenicity of clone A2b. It was discovered in the Shinitzky laboratory that the immunogenicity of leukemia antigens could be augmented by treating the tumor cells with hydrostatic pressure *in vitro* (12). Under hydrostatic pressure the lipid bilayer becomes more rigid, thereby causing aggregation and greater exposure of membrane components (13). For this reason, we applied hydrostatic pressure to A2b and other autoimmune lymphocytes with the objective of enhancing their capacity to trigger an immune response (14).

MATERIALS AND METHODS

Rats. Rats of the inbred Lewis strain were bred at this institute. They were used at the age of 2–4 months and were matched for age and sex in each experiment.

Induction of Disease. Adjuvant arthritis was induced as described (7). Briefly, rats were inoculated subcutaneously at the base of the tail with 0.1 ml of oil (incomplete Freund's adjuvant) to which was added 1 mg of *M. tuberculosis*. Adjuvant arthritis usually appeared about 13 days later. The degree of clinical arthritis was scored by estimating the swelling and redness of each of the four limbs on a scale of 1–4 as described (7, 8). The scores of each of the four limbs were added to obtain a maximum of 16 for any rat. The relative score was computed on the base of 100 for the maximum of 16. Experimental autoimmune encephalomyelitis was induced as described (2, 3). Briefly, rats were inoculated subcutaneously in a hind footpad with 25 μg of guinea pig myelin basic protein in complete Freund's adjuvant containing 40 μg of *M. tuberculosis* in 0.1 ml of oil. Experimental autoimmune encephalomyelitis usually appeared 11–12 days later. The relative clinical score of experimental autoimmune encephalomyelitis was computed by assigning 25 for tail weakness, 50 for frank paralysis of the hind limbs, 75 for paralysis of all four limbs, and 100 for a moribund state.

T-Lymphocyte Clones. The derivation, maintenance, and properties of helper T-lymphocyte clone A2b ($CD4^+$, $CD8^-$)

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have been published (8). A2b was cloned from line A2 obtained from a Lewis rat in which adjuvant arthritis was induced. Activated A2b (2×10^7 cells) caused arthritis in irradiated (750 R; 1 R = 2.58×10^{-4} C/kg) recipient rats (8). Helper T-lymphocyte line Z1a ($CD4^+$, $CD8^-$) was obtained as described (2). Activated Z1a (10^6 cells) was encephalitogenic in naive Lewis rats. It was shown to recognize an epitope from amino acids 68–88 of guinea pig or rat myelin basic protein (15). Z1a appears, indeed, to be a clone as indicated by a distinct and uniform rearrangement of its antigen receptor genes (unpublished results). A2b or Z1a was activated by incubation for 3 days with *M. tuberculosis* (50 μg/ml) or myelin basic protein (25 μg/ml), respectively, or with the mitogen Con A at 2.5 μg/ml and irradiated (1500 R) syngeneic thymus cells, which contain a population of antigen-presenting cells. The lymphoblasts were isolated by Ficoll density centrifugation and used for vaccination.

Vaccination. Activated T-lymphocyte clones or populations of lymph node cells were used. For pressure treatment, the T lymphocytes ($\approx 10^8$ cells) were placed in 1.5 ml of cold (4°C) phosphate-buffered saline (PBS) in an Eppendorf centrifuge tube as described (14). The tube was filled with fluid to avoid trapped bubbles, and a short 21-gauge disposable syringe needle was pushed through the cap of the tube to equalize pressure. The tube was then introduced into a pressure cylinder (American Instrument, Silver Spring, MD) filled with cold (4°C) PBS. Pressure was applied slowly (for 4–5 min) using a French press to a level of 1250 bars (1 bar = 10^5 Pa) for 15 min. Thereafter, the pressure was slowly released (for 4–5 min). This degree of hydrostatic pressure was found in dose–response experiments to be optimal for vaccination. About 90% of the treated cells excluded the dye trypan blue. Rats were inoculated with 1–2 $\times 10^7$ T lymphocytes three times at weekly intervals. One week after the last inoculation, or as otherwise stated, the rats were challenged with myelin basic protein (25 μg) or *M. tuberculosis* (1 mg) in adjuvant to induce active experimental autoimmune encephalomyelitis (3) or adjuvant arthritis (7), respectively, to assess the development of resistance to disease. Therapy of adjuvant arthritis was done by first inducing the disease and then treating the rats with one to three inoculations of pressure-treated cells. Vaccines were administered subcutaneously, intravenously, or intraperitoneally as indicated. The effect of vaccination did not seem to be influenced by the administration route used.

Cross-linking of membrane components was done by incubating 10^8 T lymphocytes in 5 ml of gluteraldehyde (0.3% in PBS) at room temperature for 15 min. The treated T lymphocytes were washed six times by centrifugation in 50 ml of PBS and used for vaccination as described above.

Disruption of the cytoskeleton of T lymphocytes was done by incubating the cells at room temperature for 15 min in a mixture of colchicine (10 mmol) and cytochalasin B (0.5 μg/ml) in PBS. The cells were washed and used for vaccination as described above.

Delayed-Type Hypersensitivity. Lewis rats were vaccinated and then 1 month later were challenged by subcutaneous inoculation into the pinna of 3×10^5 activated, irradiated (2500 R) T-lymphocyte clonal cells in 50 μl of PBS. Swelling of the ear was determined after 48 hr using an isotonic caliper. Histologic examination of the ear swelling confirmed the diagnosis of a delayed-type hypersensitivity reaction.

RESULTS

Table 1 shows the results of experiments in which we inoculated rats with A2b cells treated in various ways including activation by incubating the cells with antigen-presenting cells and with the *M. tuberculosis* antigen or with the Con A mitogen (there was no difference in the results between activation by antigen or mitogen). Populations of A2b were also exposed to irradiation, to hydrostatic pressure, to the chemical cross-linker gluteraldehyde, or to agents disrupting the cytoskeleton, colchicine, and cytochalasin B. Lewis rats were inoculated with the treated A2b cells and tested for their resistance to *M. tuberculosis*-induced adjuvant arthritis. Unvaccinated control rats (group 1) all developed severe adjuvant arthritis that persisted for >40 days. Rats that had been inoculated with activated A2b cells (group 2) were equally susceptible to adjuvant arthritis, whether or not the A2b cells had been irradiated (group 3).

Table 1. Treated clone A2b cells used to vaccinate against adjuvant arthritis

	A2b vaccine		Adjuvant arthritis		
Group	Cell activation	Cell treatment	% incidence (no. of rats)	Mean relative score	Duration, days
1	No	None	100 (30)	80	>40
2	Yes	None	100 (21)	75	>40
3	Yes	Irradiation	100 (12)	76	>40
4	Yes	Pressure	20 (20)	20	10
5	No	Pressure	100 (6)	75	>40
6	Yes	Glutaraldehyde	15 (12)	15	12
7	No	Glutaraldehyde	100 (12)	70	>40
8	Yes	Colchicine/ cytochalasin B	15 (12)	10	11

A2b cells were either activated or not by incubation with a mitogen (Con A) and then treated with hydrostatic pressure (1250 bars), 0.3% glutaraldehyde, or a mixture of 10 mmol of colchicine and cytochalasin B at 0.5 μg/ml. Cells (2×10^7) were administered subcutaneously thrice at weekly intervals, and the groups of rats were challenged a week later to induce adjuvant arthritis.

In contrast, rats that had been vaccinated with activated, pressure-treated A2b cells (group 4) were markedly protected: few rats developed adjuvant arthritis (20%) and those that did had very mild, transient disease. The effectiveness of pressure treatment depended on prior activation of A2b. Pressure-treated, nonactivated A2b cells (group 5) did not stimulate an immune response.

Exposure of cells to hydrostatic pressure tends to aggregate glycoproteins and other molecules associated with the lipid bilayer. We, therefore, designed experiments to learn whether chemical aggregation of A2b membrane components could also enhance the immune response triggered by A2b. Table 1 shows that treatment of activated A2b with glutaraldehyde (group 6) rendered the cells an effective vaccine. Similar to hydrostatic pressure, the chemically treated A2b cells required activation to stimulate an immune response; nonactivated glutaraldehyde-treated A2b did not substitute (group 7).

Another way to aggregate membrane components is to disrupt the cytoskeleton. Table 1 (group 8) shows that treatment of A2b with a combination of colchicine and cytochalasin B markedly enhanced the ability of A2b to stimulate an immune response. Thus, aggregation of membrane components appears to be a critical factor for the immune response.

Vaccination with pressure-treated T lymphocytes was immunologically specific; A2b-vaccinated rats resistant to adjuvant arthritis were susceptible to induction of experimental autoimmune encephalomyelitis by immunization with myelin basic protein in adjuvant (data not shown).

The effectiveness of pressure-treated clones in preventing adjuvant arthritis led us to test their ability to induce remission in rats with established adjuvant arthritis. Rats were immunized with *M. tuberculosis* and 15 days later, when all were suffering from arthritis, groups of rats were treated by a single intraperitoneal inoculation of 2×10^7

pressure-treated control Z1a or A2b cells. Fig. 1 shows that inoculation of rats with Z1a did not affect the natural course of arthritis. However, the extent of disease was markedly reduced in the rats that had been inoculated with pressure-treated A2b cells. Thus, exposure of arthritogenic clone A2b to hydrostatic pressure rendered it avirulent and turned it into an agent for preventing or treating adjuvant arthritis.

Because A2b-like clones were probably present in rats primed *in vivo*, we tested whether vaccination could be achieved using pressure-treated lymph node cells obtained directly from immunized rats. Rats were immunized either with myelin basic protein or *M. tuberculosis*, and 9 days later the draining lymph nodes were removed, and the cells were activated by culture with the T-lymphocyte mitogen Con A. The activated lymph node cells were then exposed to hydrostatic pressure and inoculated into naive rats. The rats were challenged either with myelin basic protein or *M. tuberculosis* to test their susceptibility to active adjuvant arthritis or to experimental autoimmune encephalomyelitis. Table 2 shows that activated pressure-treated control lymph node cells or lymph node cells from rats primed with *M. tuberculosis* did not immunize rats for experimental autoimmune encephalomyelitis. However, activated, pressure-treated lymph node cells from rats primed with myelin basic protein induced resistance to experimental autoimmune encephalomyelitis. In contrast to experimental autoimmune encephalomyelitis, resistance to adjuvant arthritis was induced by vaccination with activated pressure-treated lymph node cells from *M. tuberculosis*-primed rats. Hence, populations of cells containing antigen-specific T lymphocytes could be used as specific vaccines following activation and exposure to hydrostatic pressure.

Fig. 2 shows that activated, pressure-treated lymph node cells could be used to treat established adjuvant arthritis. Control lymph node cells similarly activated and pressure treated were not therapeutic.

As the antigen specificity of the vaccinating T cells influenced the specificity of the resistance to disease, it was conceivable that vaccination might involve the induction of immunity to the autoimmune receptors of the vaccine (5, 16). To test this hypothesis, we investigated the delayed-type hypersensitivity responses of vaccinated rats to particular T-lymphocyte clones. Fig. 3 shows the results of an experiment in which rats were or were not vaccinated with activated, pressure-treated clones A2b or Z1a. The rats were then challenged with *M. tuberculosis* to induce active adjuvant arthritis, and their delayed-type hypersensitivity responses were measured to A2b or Z1a cells. Nonvaccinated control rats showed background delayed-type hypersensitivity reactions to either A2b or Z1a cells, whether or not they were suffering from adjuvant arthritis. In contrast, A2b-vaccinated rats, which were resistant to adjuvant arthritis, manifested a specific delayed-type hypersensitivity reaction to A2b cells. Z1a-vaccinated rats, which were susceptible to adjuvant arthritis, did not show a delayed-type hypersensitivity reaction to A2b cells above that of background.

Table 2. Pressure-treated antigen-primed lymph node cells for vaccination

Induced disease	Primed-lymph-node-cell vaccination	Clinical disease: Incidence, %	Clinical disease: Mean relative score	Clinical disease: Duration, days
Autoimmune encephalomyelitis	None	100	88	7.5
	M. tuberculosis	100	84	7.5
	Myelin basic protein	30	40	6.0
Adjuvant arthritis	None	100	75	>40
	M. tuberculosis	0	—	—

Suspension of popliteal lymph node cells were obtained from rats that had been immunized in the hind footpads 9 days earlier with *M. tuberculosis* or myelin basic protein to induce adjuvant arthritis or experimental autoimmune encephalomyelitis, respectively. The lymph node cells were activated by incubation with Con A (2.5 μg/ml) and then treated with hydrostatic pressure (1250 bars). Groups of 20 rats were vaccinated subcutaneously with 2×10^7 cells at weekly intervals for 3 weeks. One week later, the rats were challenged with myelin basic protein or *M. tuberculosis* to induce experimental autoimmune encephalomyelitis or adjuvant arthritis, respectively.

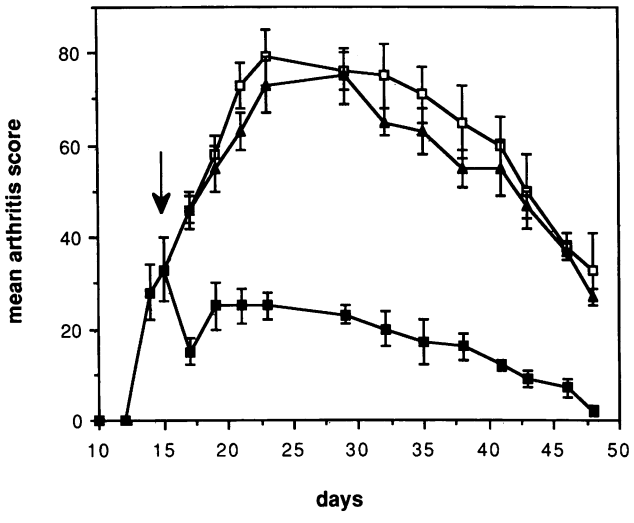

FIG. 1. Treatment of established adjuvant arthritis using pressure-treated clone A2b. Groups of 10 Lewis rats were immunized with *M. tuberculosis* in oil to induce adjuvant arthritis. On day 15 when all the rats were suffering from arthritis, the groups were treated by a single intravenous inoculation (arrow) with no cells (open squares) or with 2×10^7 Con A-activated pressure-treated Z1a (solid triangles) or A2b (solid squares) cells. Clinical arthritis was assessed. After 3 months, the rats were sacrificed and histological examination of the joints was made. The untreated rats and those treated with Z1a cells had pannus formation and destructive inflammation of the joint cartilage of the paws, while the rats treated with A2b had no signs of inflammation of the joints (data not shown).

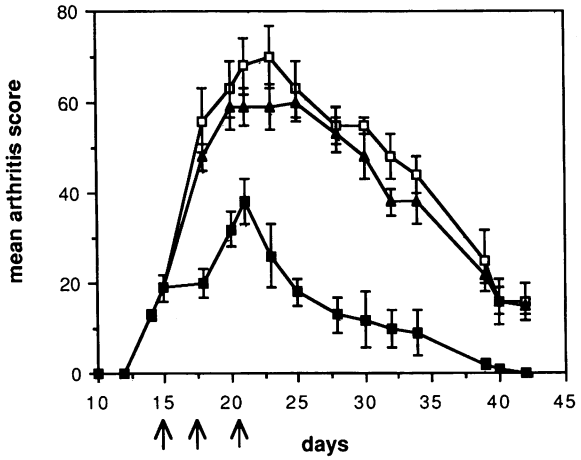

FIG. 2. Treatment of established arthritis using pressure-treated lymph node cells. Adjuvant arthritis was induced in groups of 10 Lewis rats. On days 15, 18, and 21 (arrows), the rats were inoculated intravenously with 10^7 Con A-activated lymph node cells obtained from normal rats (solid triangles) or from rats that had been immunized with *M. tuberculosis* (solid squares). Control rats (open squares) received no lymphocyte vaccination. Clinical arthritis was scored.

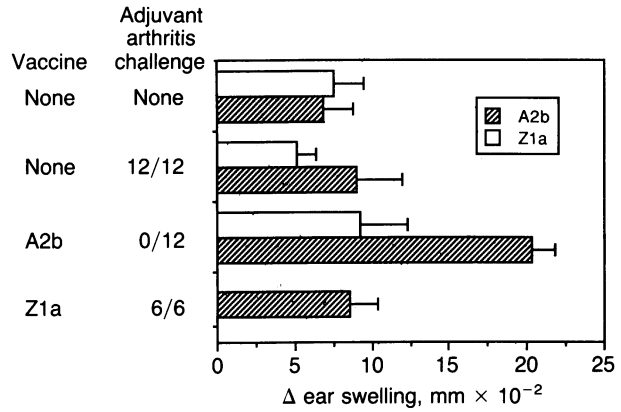

FIG. 3. Anti-idiotypic delayed-type hypersensitivity reactivity to A2b vaccine. Rats were vaccinated subcutaneously with Con A-activated, pressure-treated (1250 bars) A2b or Z1a cells. One week later the rats were challenged with *M. tuberculosis* to induce adjuvant arthritis, and the incidence of arthritis was recorded. One month after vaccination, delayed-type hypersensitivity was measured as the Δ (increase) in ear swelling after 48 hr in response to 3 × 10^5 activated, irradiated (2500 R) T lymphocytes. Error bars show the SE.

Resistance to adjuvant arthritis could be transferred from A2b-vaccinated rats to naive recipients by inoculating the latter with 10^8 donor spleen or thymus T lymphoblasts that had been activated *in vitro* by incubation with Con A. The incidence of adjuvant arthritis developing after *M. tuberculosis* challenge in the recipients of spleen cells from A2b-vaccinated rats was 1 of 12 compared to an incidence of 20 of 20 in control recipients of spleen cells from Z1a-vaccinated rats.

DISCUSSION

The results of the experiments described here demonstrate that A2b helper T lymphocytes, otherwise devoid of vaccinating capacity, could be treated so as to acquire the ability to vaccinate rats against the chronic autoimmune disease adjuvant arthritis. Similarly treated lymph node cells from antigen-primed rats could also serve to stimulate an immune response, indicating that heterogeneous populations of lymphocytes and not only defined clones, can be used to advantage.

Resistance induced by T-lymphocyte vaccination showed a high degree of specificity; cells from *M. tuberculosis*-animals vaccinated against adjuvant arthritis, but not against experimental autoimmune encephalomyelitis, and cells from myelin basic protein-primed animals vaccinated against experimental autoimmune encephalomyelitis, but not against adjuvant arthritis. Effective vaccination was found to be associated with specific delayed-type hypersensitivity reactivity to the vaccinating T-lymphocyte clone. As delayed-type hypersensitivity reactions are mediated by T lymphocytes, it is likely that effective vaccination is associated with the activity of T lymphocytes that can recognize the clonotypic marker of the vaccine. Clonotypic recognition implies recognition of the antigen receptor, a form of anti-idiotypic immunity (17).

It has been reported (18) that specific resistance to adjuvant arthritis acquired by vaccination with an untreated T-lymphocyte line could be transferred to naive rats using spleen or thymus lymphocytes. Similarly, resistance induced by activated, pressure-treated A2b could also be transferred by the lymphocytes of vaccinated rats.

Another important question is the nature of the signal carried by the immunizing T lymphocytes that induces anti-idiotypic immunity and resistance. As nonactivated T lymphocytes did not effectively stimulate an immune response, it is possible that the antigen receptor, alone or in a resting state, is not a sufficiently immunogenic signal. Activation might be needed to modify the receptor itself or combine it with other molecules dependent on T-lymphocyte activation. The finding that vaccination was potentiated by aggregation produced by chemical cross-linking of membrane components or by disrupting the cytoskeleton suggests that a multimolecular complex might constitute a functional antigenic determinant. For want of suitable strain combinations in rats, we have been unable to explore the question of whether vaccination can take place across allogeneic barriers. However, the results of preliminary studies of vaccination of mice against experimental autoimmune thyroiditis (19) suggest that vaccination using syngeneic, but not allogeneic T lymphocytes, is effective (unpublished data). This implies that, similar to other types of T-lymphocyte recognition, the response to the vaccine may be restricted by products of the major histocompatibility complex. Be that as it may, it is clear that autoimmune T lymphocytes can be used as specific therapeutic agents in experimental autoimmunity. The possible application of T-lymphocyte vaccination to clinical autoimmune diseases remains to be explored.

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