# T-Cell Vaccination Against Autoimmune Disease

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The concept that vaccination might protect against autoimmune diseases not demonstrably caused by exogenous pathogens is startling. However, not only does it have a sound rationale based on the Jerne network theory of idiotype–anti-idiotype interactions, but it has been proved experimentally with vaccination against autoimmune encephalomyelitis and adjuvant arthritis.

The perpetrators of autoimmune diseases are clones of autoimmune lymphocytes. Although this statement seems self-evident, it conveys a practical message: Arrest the virulent autoimmune lymphocytes and the disease is aborted. My colleagues and I have learned how to achieve this goal in experimental animals, using a procedure termed T-cell vaccination. My object in this article is to review our results and discuss their potential application to the bedside.

The idea that motivated our initial experiments was worked out a century ago by Louis Pasteur, Robert Koch, and their colleagues as they set out to disentangle the complexities of infectious disease, using a reductionist strategy: Isolate the etiologic agent in pure culture in vitro, and study its function in vivo. Obviously, an etiologic agent of autoimmune disease is not an invading microbe but a native citizen of the body—indeed, a member of the body's elite security force. Nevertheless, the idea seemed worth the effort: to grow in pure culture, as lines and clones, the autoimmune T cells responsible for causing a specific autoimmune disease. Perhaps the cultured autoimmune lymphocytes could function in vivo.

We, as did others, seized on the discovery that T-cell growth factors (for the most part interleukin-2) could be used to grow T cells in vitro indefinitely. Instead of raising T cells with immunoresponse specificity for egg albumin, keyhole-limpet hemocyanin, or other antigens traditionally used by immunologists, however, we chose to raise T cells specific for the basic protein (BP) of central nervous system myelin.

BP was of interest because immunization of genetically susceptible animals to BP induces experimental autoimmune encephalomyelitis (EAE), a disease characterized by mononuclear cell infiltration of the white matter and paralysis (Figure 1). It seemed likely that T cells were the perpetrators of EAE, because the disease could be transferred to immunologically naive rats by populations of T cells obtained from rats that were actively immunized to BP. Our objective was to isolate clones of anti-BP T cells in pure culture from such populations of lymphocytes. We would then investigate whether and how the clones might cause disease.

Avraham Ben-Nun, then a graduate student in my laboratory, worked out a method for selecting clones of T cells specifically reactive to myelin BP. Its success derived from the fact that T cells may be induced to proliferate at a high rate by contact with their specific antigen, presented by suitable antigen-presenting cells. When the lymph node cells of BP-immunized rats were cultured with BP, the T cells with antigen receptors that recognized BP proliferated at the expense of the other cells. In a process akin to survival of the fittest, the anti-BP clones flourished in the confines of the culture dish while all the other cells languished and disappeared.

All the anti-BP cell clones obtained in this way bore the CD4 (T4) surface marker of helper/delayed-type hypersensitivity T cells and lacked the

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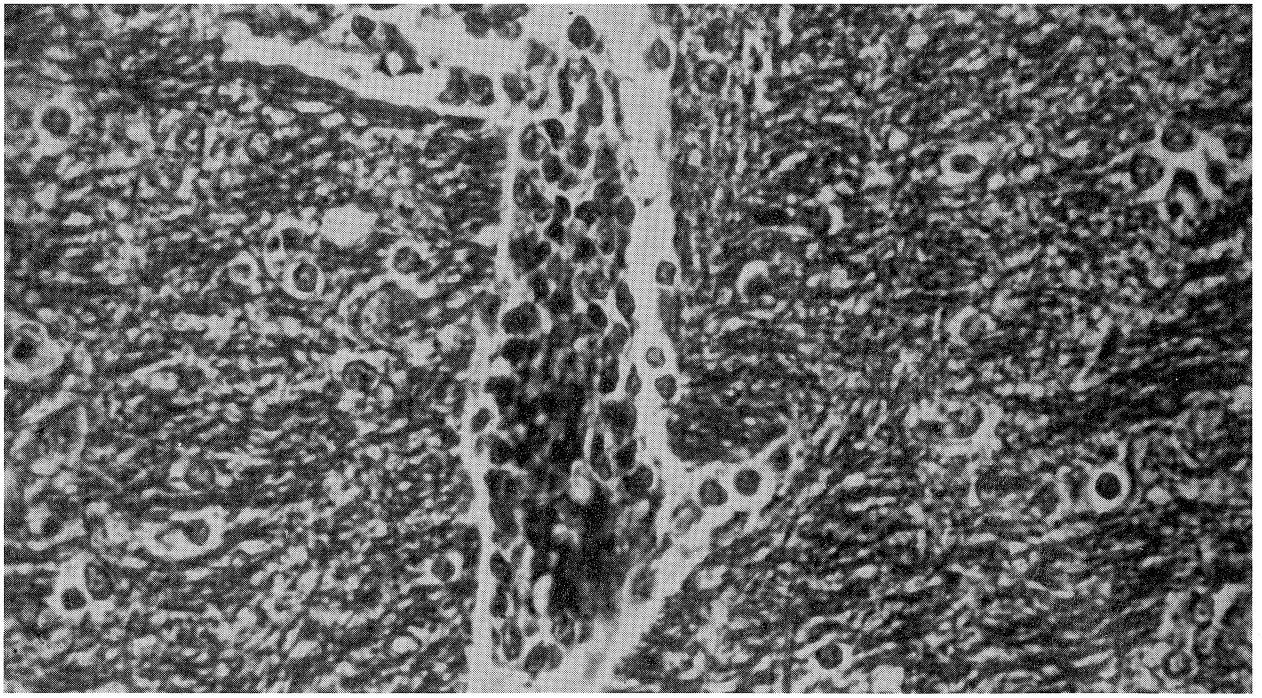

***Figure 1.*** **Perivascular infiltrate of mononuclear cells is seen in brain tissue of a Lewis rat with experimental autoimmune encephalomyelitis (EAE). A blood vessel runs vertically through the image; the vessel, and the surrounding infiltrate, are cut tangentially. Mononuclear inflammatory cells are also scattered throughout the white matter.**

CD8 (T8) surface marker of cytotoxic/suppressor T cells. These cultured clones of anti-BP T cells were indeed the etiologic agents of EAE: On intravenous inoculation, some of the cells penetrated the blood-brain barrier, accumulated in the central nervous system, and produced EAE within five days.

The disease produced by the CD4 anti-BP clones could not be attributed to classic cytolysis, which is associated with CD8 T cells. The EAE also could not have been caused by antibodies resulting from a T-B interaction; no anti-BP antibodies were detected. It seemed likely that the CD4 T cells mediated disease by triggering a delayed-type hypersensitivity reaction in the central nervous system through the activation of macrophages and other inflammatory cells. Supporting this hypothesis was the fact that the anti-BP T cells produced delayed-type hypersensitivity reactions when we injected BP into the skin. The anti-BP T cells produced inflammation wherever BP was present.

In addition to antigen-directed inflammation, we discovered that our CD4 anti-BP T cells could affect nerve conduction directly. In collaboration with Yoseph Yarom of the Hebrew University in Jerusalem, we incubated various T cells with rat optic nerves in vitro and recorded the influence of the T cells on the conduction of electrically stimulated action potentials.

The anti-BP T cells, but not T cells recognizing other antigens, were found to reversibly inhibit nerve conduction. Thus, CD4 T cells are capable of influencing myelin function, independently of their ability to trigger a local delayed-type hypersensitivity reaction. How they do this is not clear, but the phenomenon illustrates the utility of pure cultures of autoimmune T cells as probes of pathophysiology.

In addition to EAE, we have raised clones of CD4 T cells capable of causing other specific diseases: autoimmune thyroiditis in mice (a model of Hashimoto's disease) and adjuvant arthritis in rats (possibly a model of rheumatoid arthritis). Other laboratories have developed T cells that are functional in EAE in mice, in experimental autoimmune neuritis, in collagen II arthritis, and in experimental autoimmune uveitis. Efforts to isolate specific T-cell clones are being made in autoimmune diabetes and autoimmune hepatitis.

On the basis of our experiments, we felt justified in asking: Could the pathogen be turned into the preventer? We had observed that EAE produced by the anti-BP T cells was acute, and the rats either died of paralysis or recovered completely. The recovery from EAE was no less interesting than the disease, because the survivors were found to have acquired resistance against further attempts to induce EAE. Neither active immunization to BP nor inoculation with virulent anti-BP T cells produced EAE in the rats that had recovered from the initial illness.

What was the mechanism of acquired resistance? Were the rats purged of T cells that were capable of reacting to BP? Clearly not.

From the thymuses of the EAE-resistant rats we succeeded in isolating the progeny of the initially administered anti-BP T cells (confirmed by a sex chromosome marker in experiments in which male T cells were inoculated into female recipients). Inoculation of a purified culture of the T-cell isolate produced EAE in a second group of rats. Thus, we demonstrated serial transfer of EAE by a pure culture of T cells, thereby satisfying Koch's postulates for incrimination of the anti-BP T cells as etiologic agents of disease (Figure 2).

This exercise, beyond its academic appeal, demonstrated something of practical importance: After recovery from EAE, rats may continue to harbor potentially virulent T cells and yet remain healthy and resistant to further disease. Note here another analogy between infectious disease and autoimmune disease: the asymptomat-

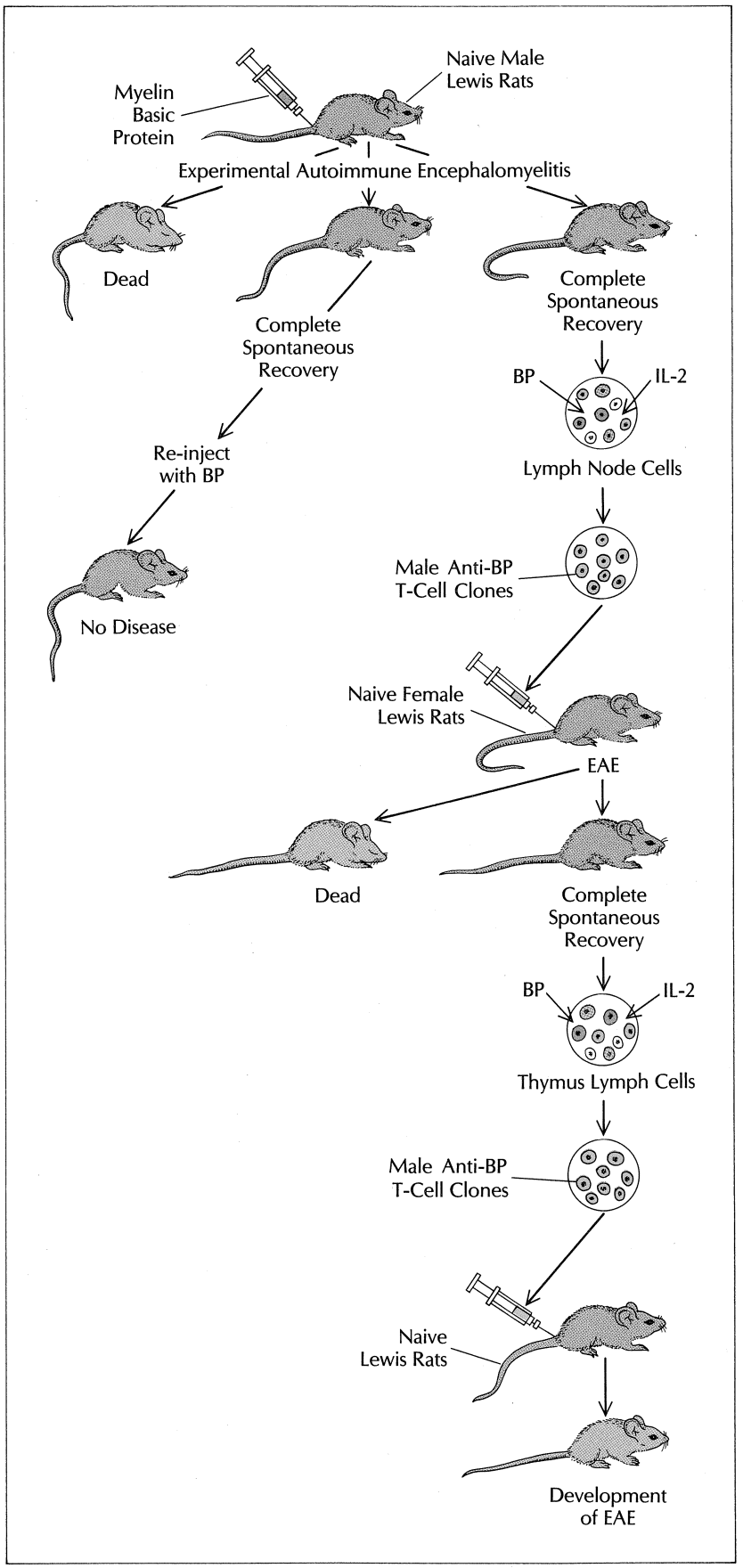


***Figure 2.*** **Serial transfer of EAE establishes that the disease is mediated by autoimmune T lymphocytes. Male Lewis rats were injected with myelin basic protein (BP), which produced EAE; the animals that recovered were EAE-immune to further injections. Lymph node cells from the recovered animals were cultured with BP and interleukin-2, causing anti-BP T cells to proliferate preferentially. The anti-BP cells were exclusively CD4 (helper) lymphocytes. Injected into naive (unimmunized) female rats, they transferred EAE. The females that recovered persisted in carrying anti-BP T cells in their lymphoid organs. These T cells were found to bear the Y chromosome, thereby proving their origin from the original inoculum of male anti-BP T cells. A purified culture of such cells was capable of transferring EAE to naive rats.**

ic carrier state. Clearly, then, the individual is capable of controlling pathogenic autoimmune T cells so that they cause no harm. Indeed, confined to a proper place and state of inactivation, autoimmune lymphocytes may be as harmless as normal flora. Achievement of such control over a patient's virulent autoimmune T cells could constitute adequate therapy of autoimmune disease.

If resistance to EAE could be induced by contact with virulent anti-BP T cells, might resistance to EAE also be induced by contact with avirulent T cells? The success of attenuated microbial vaccines was our precedent. Would vaccination with attenuated anti-BP T cells activate mechanisms capable of confining virulent anti-BP T cells?

The first vaccinations against EAE were done with anti-BP T lymphocytes that had been rendered avirulent by exposure to irradiation in vitro. Rats receiving a single inoculation of about $10^7$ anti-BP T-cell clones acquired resistance to EAE without paying the price of first contracting the disease.

Later, Evelyne Beraud, a visitor from the Faculty of Medicine of the University of Marseille, France, and Ofer Lider, then a graduate student, found that vaccination could be achieved by inoculating animals with unirradiated clones of virulent T cells, using doses of T cells below the threshold number required to produce EAE ($10^4$ or fewer cells). The rats responded to subencephalitogenic numbers of anti-BP T cells and acquired marked resistance to later administration of an otherwise lethal dose of the same anti-BP T cells. Resistance was evident about a week after vaccination and lasted without waning for as long as we tested it.

What is the mechanism of resistance induced by T-cell vaccination? How does the body actually subdue a pathogenic dose of autoimmune T cells? Experimental evidence suggests that the immune system itself inhibits the autoimmune clones by recognizing the anti-BP antigen receptors as if they were themselves antigens (idiotypes).

Nils Jerne has argued that the number of different antigen receptors in the immune system is so great (on the order of millions) that the receptors themselves must belong to the world of antigens recognizable by the totality of receptors. That the antigen receptors of B lymphocytes (antibodies) could be recognized by other B lymphocytes was experimentally demonstrable. Going a step beyond the findings, Jerne proposed that the immune system is regulated physiologically by mutual recognition among lymphocytes. The exogenous antigen sets off an immune response by intercalating itself within a network of interacting lymphocytes. The dynamics of the response, immunologic memory, and other system complexities are generated by this anti-idiotypic network.

Whether or not the Jernean network actually works as proposed, three lines of evidence are compatible with the idea that resistance to autoimmunity induced by T-cell vaccination involves anti-idiotypic immunity. First, vaccination was found to induce highly specific suppression of disease. For example, rats vaccinated with anti-BP T cells gained strong protection against EAE but not against autoimmune arthritis; conversely, T cells related to arthritis antigens protected rats against arthritis but not against EAE. T cells that recognized different peptides on the BP molecule preferentially protected rats against EAE that had been produced by immunization to the particular portion of BP they recognized. The specificity of the T-cell receptor of the vaccine to a large degree dictated the specificity of resistance induced by vaccination.

Second, vaccinated rats developed T cells that proliferated when incubated with the autoimmune T cells used for vaccination. The responding T cells could distinguish between autoimmune T-cell clones causing EAE or arthritis. This response indicated that vaccinated rats possessed anti-idiotypic T cells.

Third, resistance to a particular autoimmune disease could be transferred from vaccinated rats to immunologically naive rats by injection of the anti-idiotypic population of T cells. The experiment involved the following steps: Vaccination with anti-BP T cells in the hindfoot pads generated anti-idiotypic T cells that were transiently confined to the regional popliteal lymph nodes. Several days later, the anti-idiotypic T cells spread to other lymphoid organs. By surgically removing the popliteal lymph nodes at an early stage, we could remove the cell population containing the anti-idiotypic T cells and transfer the cells from one rat to another (Figure 3). Both the donor rats and the recipient rats were then challenged with a lethal dose of anti-BP T cells. The donor rats were found to have lost their resistance to EAE, while the recipients of the anti-idiotypic lymph node cells gained resistance.

In no instance have we suc-

ceeded in demonstrating anti-idiotypic antibodies in vaccinated rats or mice. The resistance has to be accounted for by T cells.

What kinds of anti-idiotypic T cells are generated by T-cell vaccination, and how might anti-idiotypic T cells mediate resistance to disease? By cloning the lymph node cells of vaccinated rats, Lider was able to isolate both CD4 clones and CD8 clones of anti-idiotypic T cells. In vitro, the CD4 clones stimulated anti-BP T cells, even in the absence of the BP antigen. In contrast, the CD8 clones were observed to suppress the proliferative response of the anti-BP T cells to BP. As yet we do not know whether the stimulatory clones function in the animal to enhance the disease or to inhibit it.

Hartmut Wekerle and his colleagues at the Max Planck Multiple Sclerosis Unit in Würzburg isolated from post-EAE rats a clone of CD8 T cells that specif-

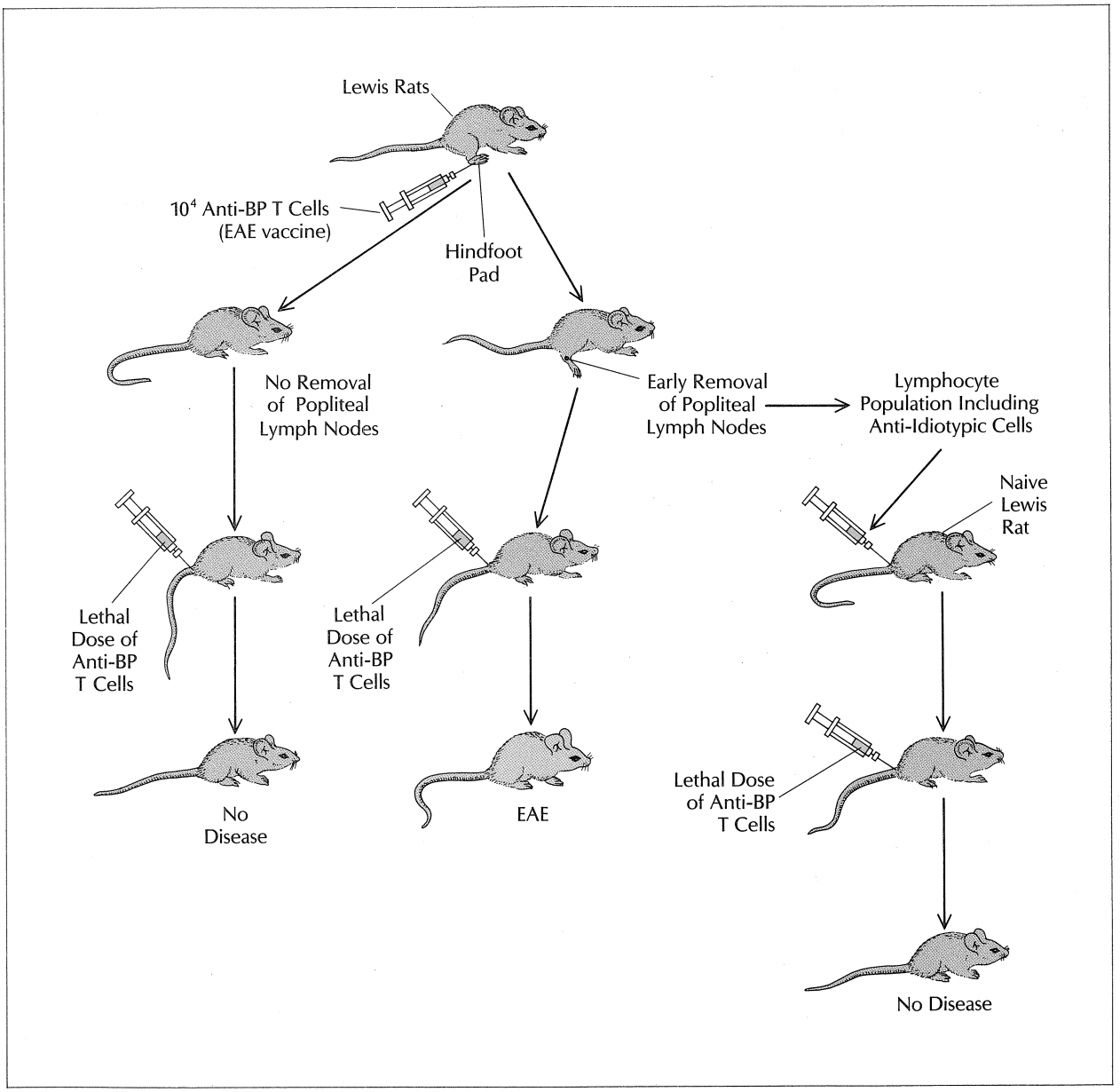


***Figure 3.*** **Role of anti-idiotypic T cells in protection against EAE is supported by an experiment in which such cells transferred immunity. Rats were vaccinated in the hindfoot pad; then their popliteal lymph nodes were removed at a time when T cells specific for the antigen receptor on anti-BP T cells had appeared in those nodes but not elsewhere. The lymphocyte population of the excised nodes conferred immunity on the recipient animals. In contrast, the animals whose anti-idiotypic lymphocytes had been removed showed no immunity despite prior vaccination. Leaving the lymph nodes in place in vaccinated animals led to lasting resistance to EAE.**

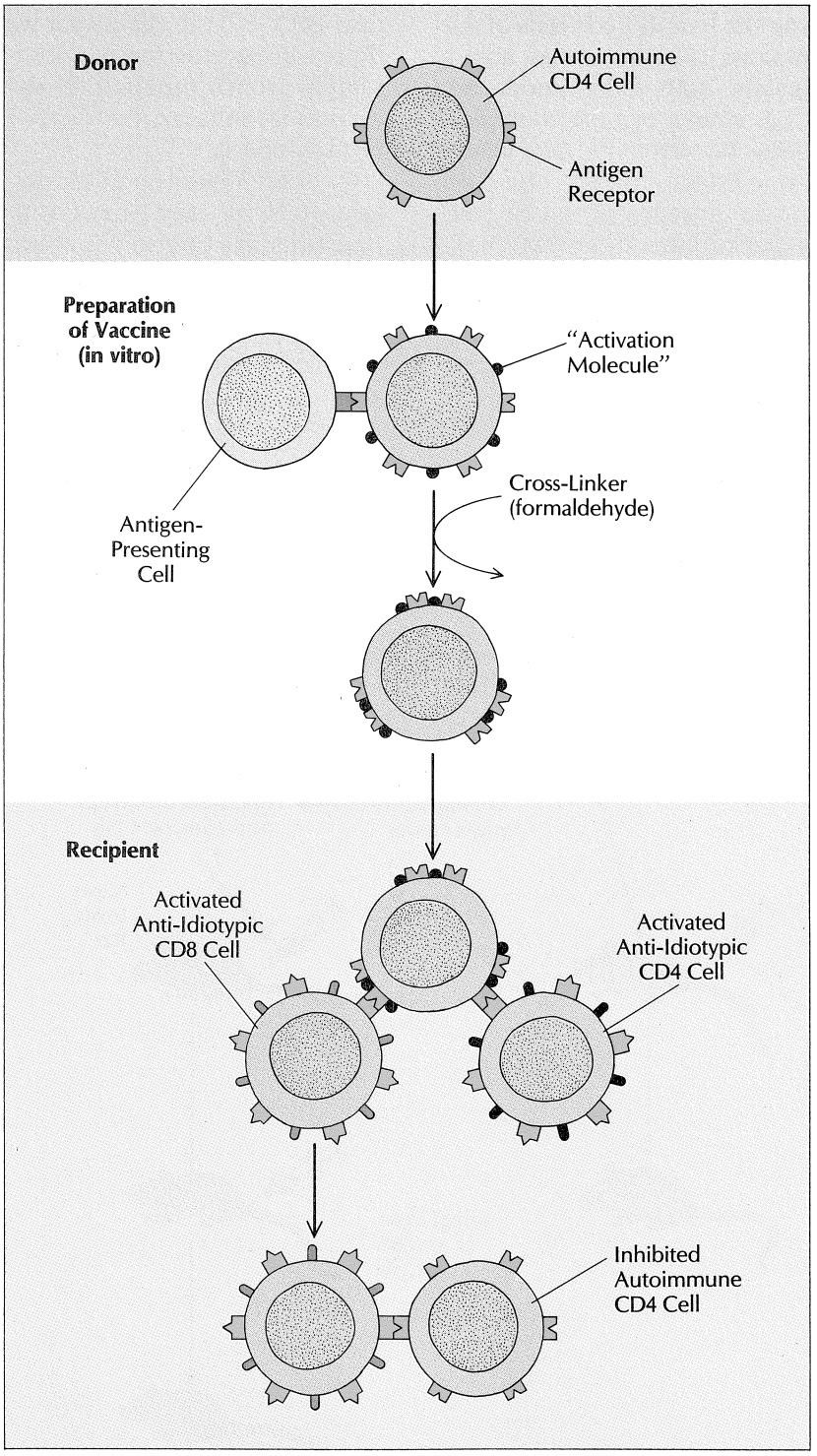


***Figure 4.*** **Preparation of a T-cell vaccine begins with isolation of autoimmune CD4 lymphocytes. Presentation of the specific self-antigen activates the lymphocytes; the actual events, not yet understood, are schematized by T-cell expression of an "activation molecule." A cross-linker, such as formaldehyde, then aggregates the cell membrane components. The activated, cross-linked cells serve as the vaccine. In the recipient animal, the cells apparently induce proliferation of anti-idiotypic T cells: CD4 (helper) and CD8 (cytolytic/suppressor) lymphocytes directed against the donor lymphocytes' antigen receptor. The CD8 cells keep autoimmune T cells in check by nonlethal means and perhaps also lytically. Actions of the anti-idiotypic CD4 cells are unclear.**

ically recognized anti-BP T cells. These anti-idiotypic CD8 T cells lysed the anti-BP T cells in vitro and protected rats from EAE in vivo. Thus, some anti-idiotypic T cells might bolster resistance to autoimmune disease by actually killing the autoimmune clones. This is unlikely to be the whole story, however. As I noted above, potentially virulent anti-BP T cells were able to persist in resistant rats. Perhaps the immune system deploys mechanisms for holding T cells in check without killing them (suppressor cytokines?).

How do anti-idiotypic T cells become immunized to the T-cell vaccine's receptor? We do not know the definitive answer to that question, but it seems that several factors are involved. The T-cell receptor may be the target of resistance, but it is itself not a compelling vaccine; the T cells must be in a state of activation to elicit an anti-receptor response. At present, a state of activation can be defined only operationally: Activation means that the vaccinating T cells had to have been exposed to their specific antigen or to a T-cell mitogen growth factor within three days of their use as vaccine (Figure 4).

Our ignorance about activation notwithstanding, its effect is impressive. Ten thousand activated anti-BP T cells vaccinate with dramatically greater effectiveness than 50 million unactivated anti-BP T cells of the same clone. Thus, the signal for vaccination not only is the antigen receptor but also must include an element induced by activation of the T cells. It makes sense physiologically for the immune system to be receptive to activated T cells; a T cell with a receptor for self-antigen will cause little harm unless the T cell is activated.

Hence, homeostasis demands that the immune system should be designed to recognize whether a potential autoimmune T cell is activated or not.

In collaboration with Meir Shinitzky of the Department of Membrane Research in the Weizmann Institute, we have also discovered that the capacity of activated T cells to vaccinate is markedly enhanced when components of the cell membrane are aggregated. Aggregation is readily brought about by treating the T cells with hydrostatic pressure or chemical cross-linkers such as formaldehyde or glutaraldehyde. In many cases, in fact, autoimmune T cells will not induce resistance to their disease unless the cells have been both activated and treated with a suitable cross-linker. Once the cells have been so treated, even their isolated membranes can vaccinate effectively. Thus, the signals required for induction of protective, anti-idiotypic immunity appear to be composed of the antigen receptor, factors related to the state of activation of the T cells, and aggregation of these cell membrane molecules.

The potential of T-cell vaccination for clinical use depends on a variety of factors—among them the ease of constructing the vaccine and the capacity of vaccination to modify the patient's already established disease. Fortunately, vaccination can be done with crude populations of lymphocytes obtained directly from immunized or sick animals. It is sufficient that the population of lymphocytes contain the relevant autoimmune cells, even in relatively low concentrations. The autoimmune T cells are selectively activated on culture with the specific self-antigen. Therefore, when the lymphocyte population is then treated with cross-linking agents such as formaldehyde or glutaraldehyde, only the activated autoimmune T lymphocytes within the cell population induce anti-idiotypic resistance.

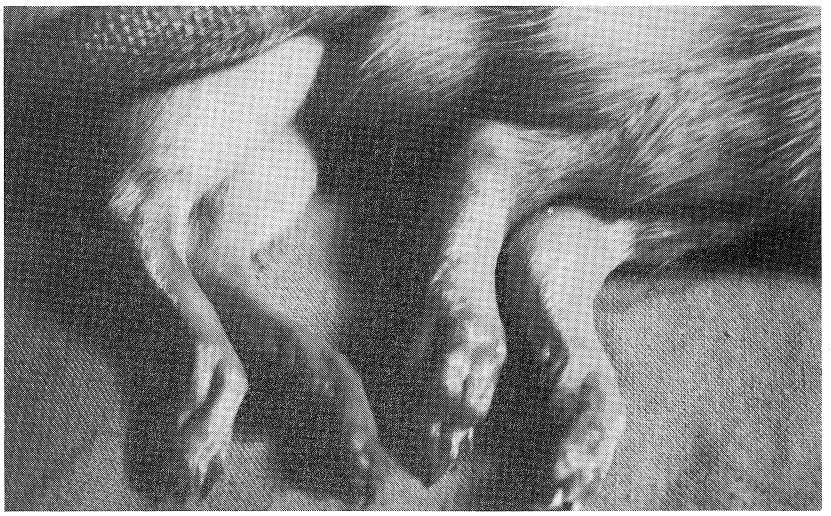

***Figure 5.*** **Adjuvant arthritis, a protracted experimental autoimmune disorder, offers opportunities to administer T-cell vaccine to animals with established disease. Here the hindpaws of a healthy Lewis rat (left) are contrasted with those of a Lewis rat in which adjuvant arthritis was induced by inoculation with killed organisms of *Mycobacterium tuberculosis* (right). The antigen provoking the autoimmune reaction in adjuvant arthritis appears to be a bacterial heat-shock protein immunologically cross-reactive with a joint self-antigen.**

For clinicians, the promising aspect of T-cell vaccination is that—experimentally, at least—it can induce lasting remission of established autoimmune disease. Hence, somewhat paradoxically, T-cell vaccination can be therapeutic rather than prophylactic. This was seen in studies of adjuvant arthritis in rats (Figure 5), an autoimmune disease triggered by immunologic cross-reactivity between a microbial heat-shock protein and a self-antigen present in the joints.

Unlike EAE, adjuvant arthritis is a protracted disease and offers an opportunity to administer T-cell vaccines as treatment after the arthritis has been established. Initial experiments in rats showed that a clone of arthritogenic T cells, suitably activated and exposed to a chemical cross-linking agent, could induce rapid and continuing remission of existing arthritis.

Subsequent experiments were done with lymph node cells obtained from arthritic rats rather than with selected, purified T clones. Here, too, activation of the cells and cross-linking of their membranes endowed them with the capacity to induce remission of established arthritis in other, genetically identical rats (Figure 6). Successful treatment of the arthritis was accompanied by enhanced T-cell reactivity against arthritogenic T clones and depression of T-cell responses to the arthritogenic antigen, which is suggestive of an anti-idiotypic response.

In view of its effectiveness in animal models, how can T-cell vaccination be put to use? Unfortunately, the answers to many important questions will become apparent only through clinical experience. Which T cells can or should be taken for

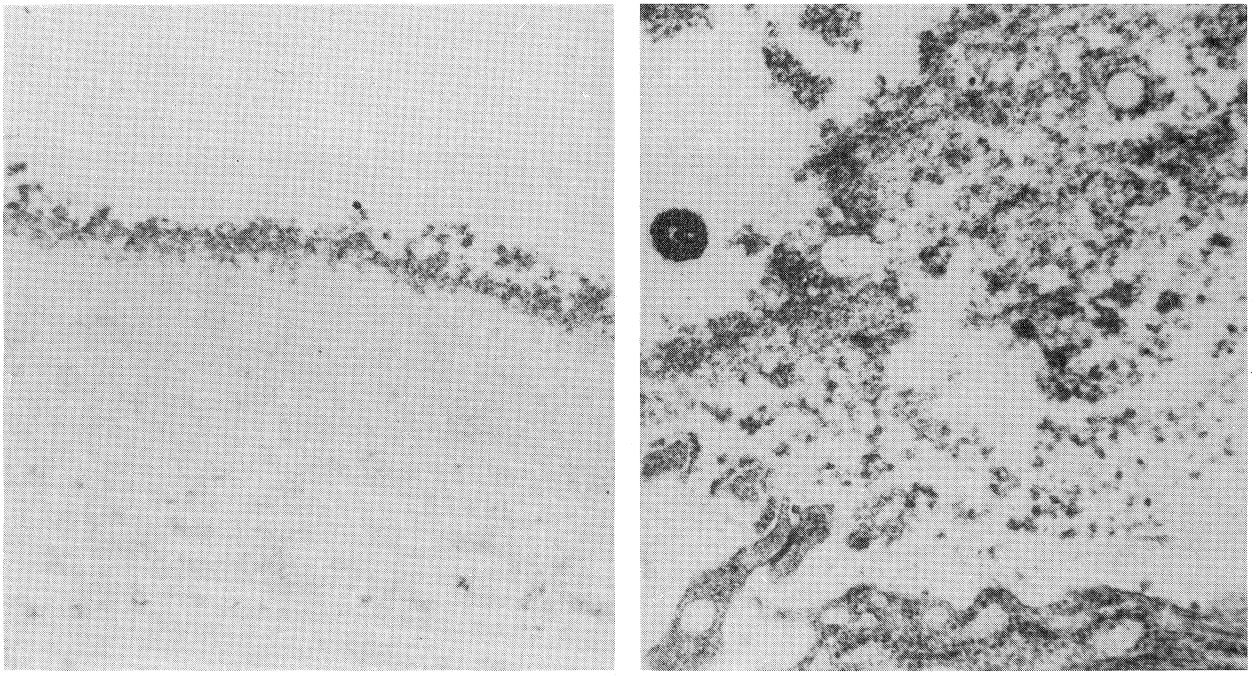

***Figure 6.*** **Remission of adjuvant arthritis after T-cell vaccination was documented by electron microscopy of the talotibial joint in vaccinated animals. A group of Lewis rats were rendered arthritic by injection of killed *Mycobacterium tuberculosis*. Lymph node cells from some of the group were activated and exposed to a cross-linking agent in vitro; the resulting vaccine was injected into other animals of the group, which went into lasting remission. Their cartilage, examined at six months, is essentially healthy (left). The unvaccinated rats developed chronic arthritis. In them, the articular surface is grossly disorganized (right), with penetration of cationized ferritin (dark spots) into the cartilage. The tissue was examined by Ritta Stanescu of the Hôpital des Enfants-Malades, Paris.**

preparation of the vaccine: intralesional cells, regional lymph node cells, or peripheral blood cells? How best can the T cells be stimulated and expanded: by specific antigen or by nonspecific mitogens? What can be done to selectively activate the autoimmune T cells when the specific self-antigen is unknown, as is the case in many autoimmune diseases? Should human T cells be cross-linked, and with what reagents? What dose of cells should be administered? Is boosting required? How best can T-cell vaccines be stored? How can one monitor whether the vaccination has taken?

A most important unknown is the hazard. No untoward reactions have been observed in many hundreds of animals treated with T-cell vaccination, but human beings might differ in their response to vaccination. Could T-cell vaccination inadvertently induce suppression not only of the autoimmune T cells but also of T cells needed to resist foreign microbes or tumor cells?

At present, autoimmune diseases are incurable, and many are life threatening or cruelly painful and disabling. It would seem, therefore, that we are obliged to confront these questions while proceeding cautiously. Indeed, a number of centers have undertaken protocols for phase I trials in several autoimmune diseases, including multiple sclerosis and rheumatoid arthritis. □

## Selected Reading


Ben-Nun A, Wekerle H, Cohen IR: Vaccination against autoimmune encephalomyelitis with T-lymphocite line cells reactive against myelin basic protein. Nature 292:60, 1981

Lider O et al: Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. Science 239:181, 1988

Sun D et al: Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T cell interactions. Nature 332:843, 1988

Cohen IR: The self, the world and autoimmunity. Sci Am 258(4):52, 1988

Cohen IR, Weiner HL: T-cell vaccination. Immunol Today 9:332, 1988

Kakimoto K et al: Isolation of T cell line capable of protecting mice against collagen-induced arthritis. J Immunol 140:78, 1988

Cohen IR: Regulation of autoimmune disease: Physiological and therapeutic. Immunol Rev 94:5, 1986

Naparstek Y et al: Effector T lymphocyte line cells migrate to the thymus and persist there. Nature 300:262, 1982

Yarom Y et al: Immunospecific inhibition of nerve conduction by T lymphocytes reactive to basic protein of myelin. Nature 303:246, 1983

van Eden W et al: Cloning of the mycobacterial epitope recognized by T lymphocytes in adjuvant arthritis. Nature 331:171, 1988