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Immunoregulation: studies of physiological and therapeutic autoreactivity by T cell vaccination

Ansgar W. Lohse[1] and Irun R. Cohen[2]

[1] 1st Department of Medicine, Johannes Gutenberg University, W-6500 Mainz, Federal Republic of Germany
[2] Department of Cell Biology, Weizmann Institute of Science, 76100 Rehovot, Israel

Introduction

Experimental models of autoimmune diseases allow the study of the pathogenesis and regulation of autoimmunity and, subsequently, the development of new therapeutic strategies. The approach of T cell vaccination in autoimmune disease does not only offer hope for a more specific and effective therapy, but in turn is helping our understanding of the regulation of autoreactive and autodestructive T cells.

Physiological and destructive autoimmunity

It was long thought that T cells with receptors for self antigens were entirely eliminated in the thymus. While this may be true for many autoreactive T cells, some autoreactive T cells seem to be part of the normal peripheral T cell repertoire [6, 7]. Hence, these cells require peripheral control mechanisms. A variety of autoreactive T cells have been isolated and characterized both in animals and in humans, but only a few of the autoantigens recognized by autoreactive T cells have so far been identified.

One of the best studied autoantigens is the basic protein of myelin (BP) [10]. This antigen is highly immunogenic in all species studied. Immunization with BP in adjuvant causes experimental autoimmune encephalomyelitis (EAE) in a variety of susceptible strains of animals [20, 22]. However, the animals usually recover after a few days. Whatever the mechanisms of recovery might be in detail, the ability to recover is evidence for peripheral control mechanisms.

BP-reactive T cells are induced by immunization with BP in adjuvant. Transfer of activated BP-reactive T cells to naive animals causes EAE. EAE can be transferred by activated T cells from diseased animals [29] by BP-reactive T cell lines and BP-reactive T cell clones [2, 4]. During EAE a high T cell reactivity to BP

Correspondence to: A. W. Lohse

can be found and limiting dilution analysis shows a high frequency of BP-reactive T cells [8, 24]. However, the presence of BP-reactive T cells does not necessarily lead to disease and various degrees of BP reactivity can be found in clinically healthy animals and, indeed, in humans.

When animals recover from EAE, T cell reactivity persists, albeit at weaker levels. After recovery of passively transferred EAE some encephalitogenic T cells persist in the animal [1, 26]. Furthermore, BP-reactive encephalitogenic T cells have been isolated from naive healthy Lewis rats, thus demonstrating that these potentially lethal autoreactive T cells are part of the normal T cell repertoire [30].

The immunogenicity and encephalitogenic properties of BP in animals suggests that it may be an important autoantigen in the human autoimmune disease multiple sclerosis. Various groups have succeeded in growing BP-reactive T cell lines and clones from patients suffering from multiple sclerosis [27, 28]. To many people's surprise BP-reactive T cells were also isolated from healthy persons, and there seemed little, if any, difference in the frequency of BP-reactive T cells between patients and controls. BP-reactive T cells are, thus, also part of the normal human T cell repertoire.

Why are autoreactive T cells to be found in the peripheral T cell repertoire and what role might they play? It is highly unlikely that autoreactive T cells are an accident of nature and serve no useful function. The answers at present are speculative. We have to learn why these cells exist and what their physiological function is. How is a normal autoreactive T cell turned into a self-destructive T cell and by which mechanisms can it be controlled again?

T cell vaccination is beginning to give us some possible answers to these questions. In T cell vaccination a clonal or oligoclonal population of syngeneic T cells is administered and the induced regulatory mechanisms can be studied. When autoreactive T cells are given as a vaccine, peripheral tolerance can be induced in a way that may reflect what is happening in the normal immune system.

Prevention of EAE by T cell vaccination

Ben-Nun et al. [2, 4] isolated a BP-reactive T cell line that was found to be encephalitogenic when preactivated in vitro. After a bout of passive EAE induced by this T cell line the animals were resistant to a subsequent attack [3]. When these T cells were rendered avirulent by preventing cell division (by either irradiation or pretreatment with mitomycin C) they no longer caused disease but the animals nonetheless acquired resistance to subsequent attempts to induce the disease [3]. Thus, the animals had been vaccinated against EAE by the application of the attenuated pathogenic agent, the BP-reactive T cell line. The analogy to microbial vaccination with its principle of induced protection by use of the attenuated pathogenic agent led to the term T cell vaccination [3, 16]. As with microbial vaccination the induced protection turned out to be specific and long lasting.

This experimental model was subject to several consistent rules. Induction of EAE by the BP-reactive T cell line Z or its subclone D9 was dose dependent: 10^7 cells caused lethal disease, 10^6 cells moderate to severe disease, 10^5 cells very

mild disease, and fewer cells caused no visible disease [5]. However, even at 10^4 cells the animals acquired protection [5]. Thus, clinical disease was not a prerequisite for immunological and clinical resistance. Both disease induction and vaccination could only be induced with preactivated cells. Administration of 10^7 resting cells from the encephalitogenic T cell lines or clones leave the animals unimpressed. They remain perfectly healthy, and perfectly susceptible to a new attempt at disease induction [5].

Various methods of attenuation of autoreactive T cells have been tried and shown to work [18]. Irradiation, chemical or physical cross-linking of the cell membranes and treatment with mitomycin C can all produce effective T cell vaccines [13]. Different methods of attenuation seem to work better with different cell lines in the various models and animal species. Uniformly the best vaccine, however, is not a vaccine in the strict sense; it is the living activated T cell. In the case of the above-mentioned encephalitogenic clone D9, as few as 10^4 activated living cells induce effective protection, but 10^6 irradiated cells and 10^7 glutardialdehyde-treated cells have to be applied for vaccination.

The principle of T cell vaccination has since been applied to a number of other animal models of both induced and spontaneous autoimmune disease [9, 11, 21]. It seems to be applicable to many experimental autoimmune diseases. This raises hope that the principle may be therapeutically useful in human autoimmune disease.

Mechanism of T cell vaccination

We are only at the beginning of understanding the mechanisms of T cell vaccination [17]. A number of cells induced by T cell vaccination have been characterized, some of them on the clonal level, but little is known about their relative contribution to the overall effect and just as little is known about the molecules involved in this cellular interaction.

The specifity of T cell vaccination has been demonstrated by a number of experiments in Lewis rats. The T cell clone A2b was shown to mediate and vaccinate against adjuvant arthritis (AA). It was, thus, possible to vaccinate animals with either A2b or D9 and assess protection from AA and EAE in the same strain of animals. Each clone prevented only the disease which it was also able to induce [17]. In addition it was shown by Lider et al. [14, 15] that vaccination with D9 induced anti-D9-specific T cells that did not cross-react with A2b. D9 and A2b are both helper T cell clones form the same strain of rats and it is likely that their main distinguishing feature is their receptor for antigen, their idiotype. These anti-clonotypic (anti-idiotypic?) T cells were of both the CD8 or the CD4 phenotype [14]. Anti-clonotypic CD8 T cells were also grown in Wekerle's laboratory [31]. His group managed to grow up enough cells to test them in vivo and show that these cells could indeed suppress EAE. Although far from proof, these experiments suggest that this cell population is, at least in part, responsible for the protection acquired by T cell vaccination. These cells were cytotoxic for the T cell clone they were raised against, but the response to this clone did not

seem to be MHC restricted. The molecule(s) recognized by this protective T cell population remain elusive.

The T cell receptor (TCR) for antigen, as explained, seems the most likely target structure for regulatory T cells induced by T cell vaccination. Two possible ways of recognition of the TCR by regulatory T cells spring to mind. The TCR might be recognized in its assembled form on the cell surface or TCR peptides might be recognized presented by MHC. As MHC restriction is what we generally expect in T cell recognition, the latter is the currently favored model. This has led to experiments attempting to vaccinate against EAE using TCR peptides. As outlined by H. Acha-Orbea elsewhere in this issue, there is very limited TCR gene usage in EAE. Suppression or control of T cells expressing these TCR gene products should control EAE. Immunization with TCR peptides has worked quite effectively in some peoples hands and the effects observed are generally similar to those seen in vaccination using whole cells [12, 32]. The underlying hypothesis is that the presentation of TCR peptides by the T cell occurs in T cell activation, but as yet there is no experimental evidence that this is what T cells do. We will have to await the isolation and cloning of anti-idiotypic T cells in sufficient number to test both their antigen reactivity in vitro and their effect in vivo. The only anti-clonotypic line characterized in sufficient detail mentioned above was not MHC restricted and, thus, not in keeping with the hypothesis underlying TCR-peptide vaccination.

In addition to clone-specific T cells, a population of T cells reactive with the activation state of the vaccinating population is induced by T cell vaccination [19]. These cells we have named anti-ergotypic T cells. They could be induced by all the activated T cells we tested and showed a proliferative response to all syngeneic and allogeneic activated T cells. Once again the response was not MHC restricted. To our surprise these cells, upon passive transfer, were able to suppress EAE [19]. These results seem to contradict the specificity of T cell vaccination mentioned above. Indeed, careful analysis showed that animals vaccinated with high doses of A2b were marginally more resistant to EAE than were naive control animals. However, this difference was minute in comparison to the protection provided by vaccination with D9 and not visible in most experiments. It was only by augmenting this effect that the protective capacity of anti-ergotypic cells could be demonstrated.

Lessons for peripheral tolerance

We referred at the beginning of this discussion to experiments by Schluesener and Wekerle [30] who succeeded in culturing encephalitogenic BP-reactive T cells from naive healthy animals. It is, thus, clear that T cells like our clone D9 are part of the normal T cell repertoire of Lewis rats. These BP-reactive T cells in the healthy animal are probably not activated, and a resting T cell can do no harm. Indeed, we have shown that as many as 10^7 resting T cells with an encephalitogenic potential have no effect [5]. It makes sense that the immune system does not bother itself with T cells that can do no harm and, thus, ignores resting T cells.

However, if BP-reactive T cells are part of the normal T cell repertoire, it must be assumed that every now and then one of these meets its antigen and

becomes activated. Nonetheless, EAE never arises spontaneously in the Lewis rat. Neither does the animal become sick, if 10^4 activated encephalitogenic cells are injected. This can only be explained by the presence of peripheral control mechanisms. In fact, the rapidity of recovery 5 days after the beginning of EAE also argues for the presence of preformed control mechanisms that are triggered by the activated BP-reactive T cells. This concept is further supported by the observation that, despite the presence of potentially encephalitogenic T cells in the periphery, active EAE can only be induced by the injection of an adequate amount of BP in a strong adjuvant. It requires a very strong stimulus to overcome the animal's natural resistance to the disease, and even then the error is corrected within a few days.

It seems likely that the same mechanisms may operate in T cell vaccination, in resistance to induced disease, and in recovery after disease induction. This resistance is T cell mediated and can be transferred to other animals by cells. We have shown that T cell vaccination induces anti-idiotypic and anti-ergotypic T cells of both CD4 and CD8 phenotype. It is conceivable that these four cell types consist of different subgroups that recognize various molecules and peptides on the cell that requires control. Additional mechanisms such as humoral reactions may contribute to the peripheral control. It thus appears that a complex network of cells is triggered by T cell vaccination, and that this network is presumably present at a very low level in the normal animal.

The role of autoreactive T cells as part of the normal T cell repertoire may lie in the induction and perpetuation of such a regulatory network [7]. The presence of the potential enemy keeps the defence on its toes. The chance is that the immune system will occasionally be challenged by autoantigens and that an autoaggressive response could thus be triggered. When this happens in the presence of certain autoreactive T cells, these cells will guide the response. The restriction of TCR usage in the animal's response to BP shows that the potential autoaggression is guided. The predictability of the autoimmune response is the immune system's chance to have the counter-autoimmune cells all ready to stop or quickly neutralize such a potentially lethal response. Thus, peripheral tolerance does not seem a case of ignorance, as conventional immunology would like to have us believe, but on the contrary a case of anticipating knowledge.

Clinical and pre-clinical applications of T cell vaccination

T cell vaccination will only become clinically useful if it can be applied therapeutically rather than prophylactically. The rapid clinical course of EAE does not lend itself to the study of therapeutic interventions. Adjuvant arthritis (AA) on the other hand runs a subacute to chronic course. Various therapeutic applications of T cell vaccination in AA have been tried and found to work. Vaccination with the T cell clone A2b had been shown to effectively prevent AA [11]. A2b was then tested as a therapeutic vaccine applied 2 to 3 weeks after disease induction, that is during ongoing disease [13]. The animals were found to make a rapid recovery and complete healing of the joints could be demonstrated histologically. Vaccination with the EAE control T cell clone did not show a significant effect

in comparison to untreated animals. Thus, just like prophylactic T cell vaccination, therapeutic vaccination has been shown to be effective and specific [16].

The use of T cell vaccination as described above requires the identification of the target antigens of autoimmune diseases and the isolation and propagation of antigen-specific T cell lines and clones from individual patients. T cells autoantigens in human autoimmune disease are mostly unknown. To make T cell vaccination more applicable to the clinical situation, we have tested the use of mixed T cell populations and non specifically propagated T cell populations. Lymph nodes were removed from animals sufering from AA and the lymphocytes stimulated nonspecifically with concanavalin A (Con A). These cells were then used as a therapeutic vaccine in other animals suffering from AA. Just like A2b, this T cell vaccine was able to hasten recovery and help healing of AA effectively [16]. Activated normal lymph node cell populations did not have this effect. We assume that the lymph nodes from diseased animals had a sufficient number of disease causing, A2b-like cells to induce specific immunoprotection.

Subsequent experiments showed that stimulation with Con A is not entirely as non specific as we had assumed, but led to an enrichment of (auto-)antigen-specific T cells [25]. Thus, the effect of Con A stimulation was more than just T cell activation, a prerequisite for T cell vaccination. The autoantigen-reactive population of T cells had presumably been preactivated in vivo and, thus, had a growth advantage upon Con A activation. After several cycles of Con A activation this advantage was lost and other cells could outgrow the autoantigen reactive population [25].

Therapeutic T cell vaccination has also been tested and found to work in the non-obese diabetic (NOD) mice. Both antigen-specific T cell lines and clones and mixed lymphocytic populations from sick animals were able to reverse insulitis and, thus, prevent the development of overt diabetes [9]. The principle has therefore, been found to be effective in both induced and spontaneous autoimmune disease, both prophylactically and therapeutically, in various animal models of autoimmune disease.

Clinical pilot trials in patients with multiple sclerosis and rheumatoid arthritis are being conduced in Boston, Leiden, London and Mainz. It is too early to comment on these studies, as the complexities of human autoimmune disease make it very difficult to know which cells should be taken from which patients at what point of time, how these cells should be propagated, attenuated and applied, how many cells should be given, how often, etc. All investigators have so far found T cell vaccination free from unwanted side effects. Most of the patients studied showed no significant immunological or clinical response, suggesting that we have not yet found the right methodology. Ongoing studies in experimental animals, primates and patients have to bridge the gap between the encouraging results in the various animal models and clinically useful applications.

References

1. Ben-Nun A, Cohen IR (1982) Spontaneous remission and acquired resistance to autoimmune encephalomyelitis (EAE) are associated with suppression of T cell reactivity: suppressed EAE effector cells recovered as T cell lines. J Immunol 128: 1450

2. Ben-Nun A, Cohen IR (1982) Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. J Immunol 129: 303
3. Ben-Nun A, Wekerle H, Cohen IR (1981) Vaccination against autoimmune encephalomyelitis with T lymphocyte line cells reactive against myelin basic protein. Nature 292: 60
4. Ben-Nun A, Wekerle H, Cohen IR (1981) The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur J Immunol 11: 195
5. Beraud E, Lider O, Baharav E, Reshef T, Friedman A, Cohen IR (1989) Vaccination against experimental autoimmune encephalomyelitis using a subencephalitogenic does of autoimmune encephalomyelitis using a subencephalitogenic does of autoimmune effector cells. I. Characteristics of vaccination. J Autoimmun 2: 75
6. Cohen IR (1986) Regulation of autoimmune disease: physiological and therapeutic. Immunol Rev 5
7. Cohen IR, Young DB (1991) Autoimmunity, microbial immunity and the immunological homunculus. Immunol Today 12: 105
8. Cohen JA, Issayan DM, Zweiman B, Lisak RP (1987) Limiting dilution analysis of the frequency of antigen reactive lymphocytes isolated from the central nervous system of Lewis rats with experimental autoimmune encephalomyelitis. Cell Immunol 108: 203
9. Elias D, Reshef T, Birk O, et al (1991) Vaccination against autoimmune diabetes with a T cell epitope of the human 65-kDa heat shock protein. Proc Natl Acad Sci USA 88: 3088
10. Hashim GA (1978) Myelin basic protein: structure, function and antigenic determinants. Immunol Rev 39: 60
11. Holoshitz J. Naparstek Y, Ben-Nun A, Cohen IR (1983) Lines of T lymphocytes mediate or vaccinate against autoimmune arthritis. Science 219: 56
12. Howell MD, Winters ST, Olee T, et al (1989) Vaccination against expeimental allergic encephalomyelitis with T cell receptor peptides. Science 246: 668
13. Lider O, Karin N, Shinitzky M, Cohen IR (1987) Therapeutic vaccination against adjuvant arthritis using autoimmune T cells treated with hydrostatic pressure. Proc Natl Acad Sci USA 84: 4577
14. Lider O, Reshef T, Beraud E, Ben-Nun A, Cohen IR (1988) Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalitis. Science 239: 181
15. Lider O, Beraud E, Reshef T, Friedman A, Cohen IR (1989) Vaccination against experimental autoimmune encephalomyelitis using a subcephalitogenic does of autoimmune effector cells. II. Induction of an anti-idiotypic response. J Autoimmun 2: 87
16. Lohse AW, Cohen IR (1989) Therapeutic regulation of autoimmunity. In: Kaplan JG, Green DR, Bleackley RE (eds) Cellular basis of immune modulation. Liss, New York, pp 451–459
17. Lohse AW, Cohen IR (1991) Mechanisms of resistance to autoimmune disease induced by T-cell vaccination. Autoimmunity 9: 119
18. Lohse AW, Cohen IR (1992) Vaccine development. (in press)
19. Lohse AW, Mor F, Karin N, Cohen IR (1989) Control of experimental autoimmune encephalomyelitis. Science 244: 820
20. MacFarlin DE, Blank SE, Kibler RF, McKneally S, Shapira R (1973) Experimental allergic encephalomyelitis in the rat: response to encephalitogenic proteins and peptides. Science 179: 478
21. Maron R, Zerubavel R, Friedman A, Cohen IR (1983) T lymphocyte line specific for thyroglobulin produces or vaccinates against autoimmune thyroiditis in mice. J Immunol 131: 2316
22. Martenson RE, Deibler GE, Kies MW, Levine S, Alvord EC (1972) Myelin basic proteins of mammalian and submammalian vertebrates: encephalitogenic activities in guinea pigs and rats. J Immunol 109: 262
23. Moore MJ, Singer DE, Williams RM (1980) Linkage of severity of experimental allergic encephalomyelitis to the rat major histocompatibility locus. J Immunol 124: 1815
24. Mor F, Cohen IR (1992) Limiting dilution analysis of the autoimmune infiltrate in experimental allergic encephalomyelitis. (in press)
25. Mor F, Lohse AW, Karin N, Cohen IR (1990) Clinical modelling of T cell vaccination against autoimmune disease in rats. Selection of antigen-specific T cells using a mitogen. J Clin Invest 85: 1594
26. Naparstek Y, Holoshitz J, Eisenstein S, Reshef T, Rappaport S, Chemke J, Ben-NunA, Cohen IR (1982) Effector T lymphocyte line cells migrate to the thymus and persist there. Nature 300: 262

27. Ota K, Matsui M, Milford EL, et al. (1990) T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 346: 183
28. Pette M, Fujita K, Wilkinsons, D, et al (1990) Myelin autoreactivity in multiple sclerosis: recognition of myelin basic protein in the context of HLA-DR2 products by T lymphocytes of multiple sclerosis patients and healthy donors. Proc Natl Acad Sci USA 87: 7968
29. Richert JR, Driscoll BF, Kies MW, Alvord EC (1979) Adoptive Transfer of experimental allergic encephalomyelitis: incubation of rat spleen cells with specific antigen. J Immunol 122: 494
30. Schluesener HJ, Wekerle H (1985) Autoaggressive T lymphocyte lines recognizing the encephalitogenic region of myelin basic protein. In vitro selection from unprimed rat T lymphocyte population. J Immunol 135: 3128
31. Sun D, Quin Y, Chluba J, Epplen JT, Wekerle H (1988) Suppression of experimentally induced autoimmune encephalomyelitis by cytolytic T-T interactions. Nature 332: 842
32. Vandenbark AA, Hashim G, Offner H (1989) Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. Nature 341: 541