American Journal of Pathology, Vol. 153, No. 3, September 1998

Commentary

The Fate of T Cells in the Brain

Veni, Vidi, Vici *and* Veni, Mori

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Since the dawn of modern immunology, immune responses within the central nervous system (CNS) have puzzled immunologists. On one hand, the immune-privileged status of the CNS has been known for a long time; along with the eye, the gonads, and the placenta, the CNS is among the few organs that accept grafted foreign cells more readily than the rest of the body.[1] On the other hand, the CNS has been known to be particularly susceptible to autoimmune disease. The first and best characterized autoimmune model, experimental allergic encephalomyelitis (EAE), which resembles multiple sclerosis (MS), is the result of an autoimmune attack by T lymphocytes on the CNS.[2] A report on EAE in this issue[3] by Bauer et al provides data of exceptional clarity that should help in understanding this apparent contradiction.

Neuroantigen-specific T cells, which can mount an autoimmune attack against the CNS, are detectable in healthy individuals. This is particularly well established for T cells that are specific for myelin basic protein (MBP), the best-characterized target antigen in the CNS. Studies using MBP-specific T cell receptor-transgenic mice suggested that these T cells normally ignore the autoantigen.[4–6] Like lymphocytes that have not encountered their antigen before, they retain a naive/resting phenotype. Such T cells dramatically change their behavior as soon as they become activated in the course of an immune response; they start to infiltrate the CNS and cause inflammatory damage there. In experimental models EAE is initiated either by immunization with a CNS antigen (for active EAE) or by *in vitro* injection of activated CNS antigen-specific T cells (for passive EAE, the model used by Bauer et al). In MS, infections with crossreactive microorganisms are thought to initiate the autoimmune attack. It remains unclear why activated myelin-reactive T cells "see" and attack the autoantigen that resting T cells with the same specificity had ignored. The information needed to promote resolution of this question pertains to the rules that govern the entry of lymphocytes into CNS tissue. This issue was addressed by Bauer et al. They injected into rats congenic T cells specific for either various CNS "self" antigens (Bauer et al, Table 1) or for the irrelevant "foreign" antigen ovalbumin (OVA). To distinguish clearly between injected T cells with a defined specificity and activation state and recruited host-derived T cells of unknown specificity and activation state, the authors took advantage of a TK-tsa-transgenic model. The detection of the TK-tsA transgene by *in situ* hybridization facilitated reliable discrimination of the injected T cells from those of the host.

T Cells Entering the CNS

Although OVA-specific T cells, irrespective of whether they were freshly activated or in a resting state, were not detected in the CNS 4 days after the injection, MBP-reactive cells (Bauer et al, Table 5) and T cells specific for three other CNS antigens (Bauer et al, Figure 2) were found to infiltrate the CNS. At first glance these data might seem to suggest that only autoreactive T cells can penetrate the blood-brain barrier and that unlike most other organs the CNS is excluded from general immune surveillance. Resting/naive T cells are known to congregate in lymph nodes, where the first encounter with antigen typically occurs, and to be sparse in nonlymphoid organs.[7,8] The degree to which these cells seek lymph nodes is determined by the expression of the lymph node homing receptor, L-selectin.[9] Activated/memory cells down-regulate L-selectin expression,[10] lose their lymph node-seeking tendency,[11] and disseminate throughout the organism,[7,8] subjecting most organs to a systematic search for their antigen. Is the CNS exempt from this search? Earlier studies by Hickey et al[12] suggested that activated T cells of any specificity can penetrate the uninflamed blood-brain barrier and that the concentration of such T cells in the CNS peaks between 9 and 12 hours

Accepted for publication July 21, 1998.

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after injection. Strikingly, only recently activated T cell blasts could enter the uninflamed CNS. These cells were found predominantly in the mesenchymal compartment near blood vessels and the meninges. Foreign antigen-reactive T cells, which were unable to "find" their antigen in the CNS, returned to baseline levels 24 to 48 hours later. The autoreactive T cells that encountered their antigen in the CNS stayed. According to these data[12] the CNS does not seem to be exempt from general immune surveillance.

The injection of preactivated CNS antigen-reactive cells results in massive cellular infiltration of the CNS, a reaction that peaked around day 6 in the case of the MBP-specific cells used by Bauer et al (Bauer et al, Table 3). Although the specificity of the T cells that initiate this infiltration is highly defined in this experimental setup, the nature of the T cells that constitute the infiltrate had been a matter of controversy before these experiments. It is, however, of particular relevance for MS and other autoimmune diseases in which the target antigens are not known. The study of T cells that infiltrate the target organ is among the most promising approaches to identifying T cells that mediate these diseases. It remained unclear whether the infiltrating cells are the injected cells themselves or progenitors of them that arose locally through antigen-driven proliferation. Alternatively, the infiltrating cells might have originated in the host and been recruited to the inflamed site, in which case recruitment might be random or might primarily involve CNS antigen-specific cells. Additionally, it has been unclear whether the rules that seem to govern leukocyte entry into the noninflamed CNS also apply to the inflamed organ, perhaps permitting the entry of naive T cells and resting memory cells in addition to T cell blasts.

The experiments done by Bauer et al provide answers of exceptional clarity to these questions. They showed that in the early stage (day 4) of EAE caused by TK-tsA-transgenic, MBP-specific cells, up to 50% of the T cells in the CNS are transgene-positive and that their frequency drops to approximately 25% at the peak of the disease on day 6 (Bauer et al, Table 3). These cells did not proliferate significantly in the CNS, as the cell cycle marker "proliferating cell nuclear antigen" was rarely detected in these T cells (Bauer et al, Figure 1). The remaining 50% to 70% of the T cells in the infiltrate were clearly identified as host-derived. Of these, most must have been bystander cells with irrelevant antigen specificity, because when OVA-specific transgenic cells were coinjected, these were also detected in high frequency in the cellular infiltrate (Bauer et al, Table 5). When these OVA-specific cells were freshly activated, they were recruited to the inflamed CNS more efficiently than when they were resting (Bauer et al, Table 5). These data show clearly that acute inflammatory T cell infiltrates in EAE are made up of a mixture of autoantigen-reactive and irrelevant antigen-specific bystander cells. T cell receptors in autoimmune lesions are therefore also likely to consist of these two components. According to these data from Bauer et al, prevalent V gene utilization in lesions can reflect peripheral events not necessarily related to the autoimmune process. Were a superantigen, for example, to activate and induce clonal expansion of certain V gene-bearing cells that are not CNS antigen-specific, these cells should still preferentially migrate and accumulate in the CNS, if only because they are activated. Although the accumulation of V gene-bearing cells may indicate superantigen-driven autoimmunity, it alone does not provide clear evidence of it.

T Cells Dying in the CNS

Once T cells have been sensitized to an antigen they tend to combat it until the antigen is cleared. Clinical examples are the frequently therapy-resistant rejection of transplanted organs and the inexorable progression of autoimmune diseases. In EAE one might also expect the CNS antigen-reactive T cells to operate under this "*veni, vidi, vici*" principle (Julius Caesar's famous "I came, I saw, I conquered" message sent to Rome after victory in battle) because there is abundant autoantigen available in the CNS to "see" the CNS antigen-reactive T cells and stimulate them to win. If this general response pattern characterized EAE, the autoimmune attack would perpetuate itself and continue until the target tissue was destroyed. Yet EAE comes to a spontaneous halt, typically in less than a week. In most rodent models EAE is monophasic, recovery is complete and permanent, and the animals even become resistant to the re-induction of EAE. The rat model studied by Bauer et al falls into this category. Why this autoimmune disease is self-limiting has puzzled researchers since the model was introduced. In addition to being a paradigm for autoimmune disease, monophasic EAE is a powerful paradigm for peripheral tolerance mechanisms that rapidly correct the mistake in self/non-self discrimination that causes the disease. Even in murine models in which chronic EAE develops, there is frequently complete recovery from the first episode of the disease before a relapse occurs and subsequent disease episodes also tend to be remitting.[13] Why is this autoimmune disease characterized by a self-limiting course, halting spontaneously and then starting again? Counterregulatory immune mechanisms have been implicated.[14] Exhaustion of the T cell response and apoptosis from "overwork" also seemed to be plausible explanations.[15] Alternatively, it is conceivable that the T cells lose their function, becoming anergic and eventually dying, when they encounter the antigen under suboptimal conditions of antigen presentation; microglia and astrocytes, which can present antigen but do not express appropriate costimulatory molecules, have been implicated in the inactivation of the autoreactive T cells.[16,17] Similarly, the abundant apoptotic T cells that can be detected in EAE lesions have been thought to represent autoreactive T cells that die because of such unfavorable antigen recognition or overwork.[18,19] The prevalent view was that T cells have to come and see before they die (*veni, vidi, mori*). The data from Bauer et al challenge this view.

Bauer et al clearly show that not only the T cells reactive to various CNS antigens (Bauer et al, Figure 2) but also those reactive to the foreign antigen OVA (Bauer et

al, Table 5) undergo apoptosis in CNS lesions and that the host-derived T cells, in addition to the injected MBP-specific cells, die (Bauer et al, Table 4). Because the CNS antigen-specific T cells induced EAE they must have recognized autoantigen in the CNS and must have secreted cytokines there. Apparently, though, they did not proliferate before they died, as very few of them expressed the cell cycle marker 'proliferating cell nuclear antigen'. Antigen recognition on resident antigen-presenting cells (astrocytes and microglia, which deliver costimulatory signals deficiently and are therefore implicated in the induction of anergy and apoptosis[16,17]) did not augment the rate of apoptosis in MBP-specific T cells, a fact that was elegantly shown with major histocompatibility complex-mismatched chimeras. Overall it seems as if the mere entry of a T cell into the neurodermal parenchyma would suffice to eventually activate the apoptosis program in a T cell; in the parenchyma of the inflamed CNS, *veni, mori* seems to apply for T cells. By showing that all T cells, not only the antigen-specific ones, die, these data substantiate that the CNS is an immune-privileged organ and suggest a mechanism for it. Fas-FasL interactions have been shown to mediate the immune privilege of the eye.[20] While it is tempting to implicate the same molecular mechanism for the CNS, the rate of T cell apoptosis in the CNS was not found to be reduced in mice that are genetically deficient for Fas or FasL.[21] Several other apoptosis pathways are discussed in Bauer and colleagues' paper.

In light of all these findings, the immune privilege of the CNS could be defined as follows. The CNS does not prevent peripherally activated memory cells, T cell blasts in particular, from entering and surveying it. Naive T cells and resting memory cells might not be able to penetrate the noninflamed blood-brain barrier. If the peripherally preactivated T cell blasts encounter "their" antigen ("foreign" or "self") in the CNS, they can induce a vigorous inflammatory response: T cell blasts, macrophages, and, to a lesser extent, resting T cells (Bauer et al, Table 5) are now recruited through the inflamed blood-brain barrier and engage in a delayed type hypersensitivity (DTH) reaction. The duration of this T cell-mediated DTH reaction is stringently controlled, however, by the indiscriminate killing of all infiltrating cells after a couple of days. In this way the immune surveillance and protection of the CNS is warranted yet chronic inflammatory reactions that could cause permanent damage to the CNS are prevented. This precaution might be seen as an understandable requirement because CNS functions are more sensitive to cellular injury than are those of most other organs.

T Cells Surviving Entry into the CNS

There is a further striking and novel observation reported in the paper by Bauer et al, namely that apoptosis of T cells in the CNS is confined to the neuroectodermal parenchyma and is not seen in the connective tissue compartment of this organ, the perivascular space and the meninges. The latter is where T cell blasts preferentially enter for immune surveillance and where they leave if they do not encounter antigen.[12] The autoimmune lesions in MS and in chronic EAE are usually perivascular. It is tempting to speculate, therefore, that this connective tissue compartment represents the germinal center for chronic immune and autoimmune processes. For example, whereas the majority of the autoreactive T cells die in the neuroectodermal parenchyma, which leads to recovery from the initial EAE attack, the autoreactive T cells in the perivascular compartment might become stimulated to engage in clonal expansion by the endogenous antigen, giving rise to a new generation of effector cells. Determinant spreading, a process during which target-organ-specific autoreactive T cells with different specificities become activated,[21–25] also might occur in the connective tissue compartment. Should the effector cell mass generated during the second wave response suffice to cause disease (induction of EAE is strictly dependent on the injection of a minimal number of CNS antigen-reactive T cells), a relapse would occur. As in the primary episode of the disease, the cells that enter the neuroectodermal parenchyma will die and the second episode of EAE will also become self-limiting. In this manner relapses and remissions of the disease could be caused by the fluctuation of new waves of T cell responses generated in the CNS mesenchyma followed by their decimation in the parenchyma. It is tempting to speculate that chronic autoimmune disease of the CNS will occur only in those individuals in whom, for various genetic and somatic reasons, the amplifying mesenchymal reaction prevails or in whom the counter-regulatory apoptosis in the parenchyma is defective. However, the apoptosis pathway might be the more common one. Only a few rodent strains are sensitive to induction of even monophasic EAE and it took decades to identify the select strains and protocol combinations that permit induction of chronic relapsing EAE.

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