Commentary

Some Misconceptions about Understanding Autoimmunity through Experiments with Knockouts

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Experimental autoimmune encephalomyelitis (EAE) has served as a prototypic model of T cell–mediated, organ-specific autoimmune disease, and as a useful model for the human disease, multiple sclerosis (MS) (1). Frei et al. (2) demonstrate that in two strains of mice with a double knockout, where both TNF-α and LT-α are inactivated, that EAE may develop. The disease in these double knockout mice progresses in an apparently typical fashion, concordant with what is observed in the usual inbred strains of mice where EAE is induced: there is clinical paralysis, and histopathology reveals intense perivascular and parenchymal infiltration with CD4+ T cells and demyelination. They conclude, and I agree, that the results are surprising, given the large body of information suggesting that TNF-α and LT-α are important in the pathogenesis of EAE and MS. However, before accepting their ultimate conclusion that, "these results indicate that TNFα and LT-α are not essential for the development of EAE," it is worthwhile to consider the limitations of the first-generation knockouts that have been employed in their study. Certain misconceptions have arisen concerning the interpretation of experiments with these contemporary knockouts. This is especially true when trying to understand the role of critical effector molecules like cytokines, in the development of complex phenotypes, like the paralysis and inflammation seen in EAE. Many of these cytokine molecules have diverse biological activities, and many of the functions of these molecules can be duplicated by other cytokines. Thus, in animals with disrupted or "knocked out" cytokine genes, one may expect many diverse changes in several physiological processes, and one might find that after all is done, that another gene and its product can replace the function of the gene that was disrupted.

Contradiction: TNF-α and LT-α Are Critical in the Development of EAE, Yet Disease Occurs in the TNF-α–LT-α Double Knockout

There is abundant evidence that TNF-α and LT-α are critical in the development of EAE, and in the human disease, MS (3–12). Both TNF-α and LT-α mRNA and protein are in the central nervous system in acute EAE (3–6). T cell clones, reactive to myelin basic protein, are more capable of mediating EAE, when they produce higher amounts of TNF-α and LT-α (7). Blockade of clinical paralysis in EAE has been successful with anti-TNF antibodies (8, 9) or soluble TNF type I receptors (10, 11). Reversal of EAE is seen with altered peptide ligands of myelin basic protein that reduce production of TNF-α (12, 13). Reduction of TNF-α with type I phosphodiesterase inhibitors like the antidepressant, Rolipram, also leads to the reversal of EAE (14, 15). Relapsing attacks of paralysis in EAE, which can be induced with superantigens, are blocked with anti-TNF (16). TNF is produced in high amounts by glial cells in strains that are susceptible to EAE, but not in resistant strains (17). Demyelination is mediated in vitro in oligodendroglial cultures by TNF-α and LT-α (18). Overexpression of TNF-α in the central nervous system leads to demyelination (19). This experiment in a transgenic mouse with the TNF-α transgene expressed in the central nervous system, stands in contrast to the double knockout mice used here, where the LT-α and TNF-α genes are disrupted throughout the animal, not only in the central nervous system. Injection of TNF-α can trigger relapses of EAE (20, 21). All these experiments in EAE, reinforce the findings indicating that TNF-α and LT-α play a pathogenic role in MS: TNF-α and LT-α are found in demyelinating lesions in the brains of MS patients, and increases in TNF can be seen in the spinal fluid before relapses (22).

Reconciliation of the Data On the Role of TNFα in Demyelinating Disease

Cytokines may exert several, even opposing effects at distinct points, both anatomically or temporally during an immune response. Produced and released naturally during an immune response, cytokines will be regulated, and their action will usually only target cells at the site of production. Conversely, systemic administration of cytokines inevitably cannot be under such control, and thus adverse rather than physiologic effects may ensue. Thus, cytokines often produce paradoxical effects when delivered systemically, rather than via effector cells at the site of pathology in situ: TNF-α may inhibit demyelinating disease, for instance, in Theiler's virus induced demyelination (23). Likewise in EAE, delivery of TNF-α by a recombinant vaccinia virus inhibited EAE (24). Similarly with Th2 cytokines, systemic delivery of IL-4 worsens EAE (25), while local delivery of IL-4 via T cell clones ameliorates disease (26).

There are numerous effector molecules in EAE. It is clear that IL-6 (27), nitric oxide (28, 29), and TNF-α may all play a role in the immunopathology of EAE and MS.

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Given the redundant function of these molecules, it is not surprising that inactivating one or more of them, may still not influence a change in phenotype.

The remarkable redundancy in cytokine function is elegantly demonstrated in mice with a transgene for a TCR for myelin basic protein and an inactivated RAG-1 gene. In these mice EAE still develops without Th1 T cells. Examination of their brains reveals IL-4 production without evidence for TNF-α. Thus even without Th1 cytokines, EAE can still develop with the appearance of Th2 cytokines in active lesions (Lafaille J, J. Van de Keere, J. Baron, W. Haas, and S. Tonegawa, manuscript submitted for publication).

Given these intricacies and redundancies in cytokine pathways, it may not be accurate to claim that TNF-α and LT-α are not essential for EAE in normal animals. As Frei and colleagues write, "Alternatively, in their absence other cytokines may compensate for the defect." (27). I would support this interpretation, and I would inquire about what other cytokines were expressed in these inflamed brains. Were other Th1 cytokines expressed, or were Th2 cytokines present? Indeed gamma interferon transcripts were found in the brains of animals showing EAE with TNF-α and LT-α inactivated. Paradoxically perhaps in inbred mice without disrupted genes, administration of gamma interferon inhibits EAE, while antibody to gamma interferon enhances EAE (27, 30).

There are major abnormalities in many of the new strains of mice with disrupted (knocked out) genes using the contemporary technology. Often, the genes under study are inactivated during the entire life of the organism, and are inactivated throughout the organism. In these TNF-α– and LT-α–deficient mice there is abnormal spleen architecture, blood lymphocytosis, absence of lymph nodes, and functional defects in T cell physiology (31). Are these appropriate conditions to study gene function and make conclusions about the role of these genes in autoimmunity?

Perhaps the next generation of knockout animals with spatial and temporal control of the inactivated gene will be more useful (32). Wilson and Tonegawa write: "The major drawback of the current gene-knockout technology, as applied to the brain, is the lack of regional and temporal specificity." (32). Identical problems also confound studies on immune function with the current knockouts for cytokine genes, now being used. Work with the current generation of knockouts in EAE show that disease develops well when gamma interferon is inactivated (33), when IL-4 is inactivated (34), and when TNF-α and LT-α are inactivated (2). Obviously in these knockout mice other cytokines and mediators may then assume the functions of the deleted products of the inactivated genes. Future studies involving knockouts need to take these issues into consideration. Given new technologies involving microarrays, it is now possible to screen for a wide variety of cytokines, chemokines, metalloproteases, adhesion molecules, and other critical mediators on a single sample of RNA from the brain of a mouse with EAE (35). Comparison of the array of mediators transcribed in such knockout mice with EAE to other mice without the disrupted genes, who nevertheless develop EAE, will give further clues to how alternative pathways are utilized to achieve a classical phenotype. The concept that there is a single mediator ultimately causing pathology in a phenotype as complex as EAE, is probably flawed. The culmination of a complex sequence of pathological events results in disease. Studies with the current generation of knockouts reveal the redundancy of certain mediators, like TNF-α and LT-α, in complex pathophysiological events.

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