A Phenotype or Not: Targeting Genes in the Immune System

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Ever since molecular analysis began to prevail in immunology and more and more molecules seemingly involved in the control of the development and function of the immune system were being discovered, inactivation of specific genes in the mouse germ line (and in the resulting adult mouse) (*1*) had been a scientific dream.

The mouse is the most popular experimental animal in immunological research, and as a result the development, cellular composition, and functional activities of its immune system have been studied in much detail. Thus, targeted mutation in the mouse of genes encoding proteins of immunological interest is an ideal way to directly assess the function of those molecules in vivo. Inactivation of particular genes in the immune system has an additional attraction: the system is "redundant" in the sense that a mouse can live without it under sterile conditions. A mutational analysis is therefore not hampered by lethal phenotypes—a major problem in other contexts.

I realize from a grant application written early in 1988 how uncertain it (or better, I) still was then whether specific gene inactivation through homologous recombination (gene targeting) would ever become feasible in the mouse in practice. Now, in early 1992, due to the pioneering work of investigators outside immunology (*1*), the creation of "knock-out" mice is a routine technique, and we can begin to assess its usefulness and its future applications.

Is gene targeting worth the effort for the analysis of the immune system? Undoubtedly, yes. Who could have been sure, before knock-out experiments were done, to what extent the generation of T lymphocytes depends on the presence of major histocompatibility antigens and that these molecules are otherwise dispensable (*2*)? Or that, in the development of antibody-producing cells (B lymphocytes), membrane expression of antibody heavy chains at an early stage is required for the cells to survive and to restrict antibody expression to a single specificity (*3*)? Or that the recombination-activating genes *RAG-1* and *RAG-2* are indeed critical for immunoglobulin and T cell receptor gene rearrangements but are not noticeably required for the development of the brain (*4*)?

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So far all of the published experiments except one (*5*) in which genes specifically expressed in the immune system were inactivated by targeted mutation have yielded distinct, although not necessarily expected, phenotypes. This is also true so far for the soluble mediators called interleukins (ILs), for which in vitro experiments had predicted substantial functional redundancy. Indeed, thymic development appeared undisturbed in animals deficient for either IL-2 or IL-4 (*6*), although both lymphokines had been suspected to play a role in this process. Breeding experiments will show whether the two proteins can substitute for each other in this context or whether they are not needed altogether. However, either lymphokine clearly displays functional activities that cannot be replaced by others. IL-4–deficient mice, for example, are unable to produce immunoglobulin E antibodies. For yet another interleukin, IL-10, the major phenotype that has been identified is a reduction of the body weight by one-third (*7*)—certainly not what we had expected. How or whether this can be related to a functional deficiency in the immune system remains to be determined, but I am convinced that, as in the case of IL-2 and IL-4, IL-10 deficiency irreplaceably affects some immunological function.

A gene-targeting experiment that does not result in a clear phenotype can be an agonizing experience for the experimenter. First, one has to make sure that the gene in question has indeed been functionally inactivated. This can be a difficult task; complications can arise from alternative RNA splicing, unforeseen transcriptional start sites, and so forth. Second, and more important, the search for a phenotype is boundless until one has found it; and if one finds a minor effect, how can one be sure that this reflects the essential defect? We are precisely in such a situation in our analysis (*5*) of the function of immunoglobulin D—an antibody isotype expressed in a developmentally controlled fashion in B cells of mouse and man and suspected to be involved in quite a variety of regulatory processes. Even in homozygous mutant mice that lack immunoglobulin D expression altogether, a major phenotype remains to be discovered.

Thus, knock-out experiments quickly tell us what a particular gene is *not* required for, but may not easily disclose its unique function. I would extrapolate that such a function indeed exists for most genes from the fact that most targeted mutations in immunologically relevant genes have produced clear phenotypes.

The successful identification of these phenotypes relies on the sophistication with which the immune system of the mouse can be analyzed. In the absence of such sophisticated analysis, the identification of gene function through knock-out experiments will be often difficult if not impossible, and genes may be erroneously considered redundant when the right question is not, or cannot be, asked.

The mouse strains generated through gene targeting in the immune system are of eminent practical value because they allow an assessment of the role that individual components of the system play in immunological defense and autoimmune disease. Mice selectively lacking B cells (*3*), helper or cytotoxic T cells (*2*, *8*), certain lymphokines (*6*), or all lymphocytes (*4*) are already available, and this list is undoubtedly going to increase rapidly. Such animals, as well as crosses between them or to strains genetically prone to autoimmune and other diseases, should have a major impact on medical research.

Gene targeting not only allows gene inactivation and deletion, but can also be used for the targeted introduction of both subtle mutations (*9*) and new genetic elements, as well as for gene substitutions (for example, of mouse genes by their human counterparts), and it often represents the only way by which the function of a newly discovered gene can be identified. These many applications will ensure that this approach will be uniquely helpful in future work on the immune system as such and its involvement in disease.

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