Tracking the Lost I-J Suppressor Region

New evidence suggests that two genes, one located in the mouse MHC and one located elsewhere in the genome, are needed to make suppressor molecules

About a year and a half ago the I-J suppressor region of the major histocompatibility complex (MHC) turned up missing. This issue of *Science* carries a research article* by Colleen Hayes of the University of Wisconsin–Madison and her colleagues that may help to clear up the mystery.

The I-J region is of interest because it is supposed to carry the genes for an important type of immune regulatory molecule that is made by the suppressor class of T cells and inhibits antibody production by B cells. The I-J molecules have been detected antigenically on the surfaces of suppressor T cells, where they may form part of the long-sought T cell receptor for antigen, and as soluble factors secreted into the culture medium. Knowledge of the biochemical nature of suppressor molecules would contribute a great deal to unraveling the myriad regulatory interactions in which T cells participate. But the suppressor molecules have not been isolated and characterized completely, and remain somewhat controversial.

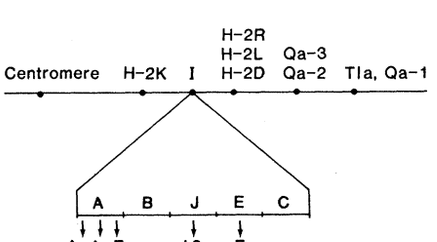
Classical genetic studies mapped the genes encoding the suppressor molecules to the I region of the mouse MHC, which contains the genes coding for the class II histocompatibility molecules that regulate the activities of immune cells. In particular, the suppressor region was localized to a position between the I-E and I-A subregions and given the designation I-J. (The various MHC gene regions were given alphabetical designations.)

The past 3 years have seen much progress in determining the structures of the other histocompatibility genes and their protein products (*Science*, 27 May 1983, p. 937). About 2 years ago, Michael Steinmetz, who was then working in Leroy Hood's laboratory at the California Institute of Technology and is now at the Basel Institute of Immunology, identified a probe of cloned DNA that extended into the I-J region. The hope was that this probe would permit the isolation and characterization of the suppressor molecule genes, thus revealing at last the nature of the suppressor molecules.

But then came the surprise. By the end of 1982, Steinmetz and Hood found that there are at most 3.4 kilobases (kb) of DNA between the I-E and I-A regions. Moreover, the 3.4 kb contain about half of one of the I-E region genes (E_β). The I-E and I-J molecules cannot be identical because they are located on different cell populations and recognized by different antibodies. It was beginning to look as if the suppressor genes could not be located where genetic mapping studies said they should be. The I-E and I-A regions might even be contiguous.

More recently, Hood and Joan Kobori, also of Caltech, have narrowed the region between I-E and I-A to a maximum of 2.0 kb, mostly consisting of E_β sequences. "We were all left with the dilemma of how to account for the two series of results," Hayes says.

There are a number of ways of explaining the discrepancy between the genetic and molecular results, including the possibility that the suppressor molecules might be artifacts. In fact, at one time an additional genetic region, designated I-B, was supposed to lie between I-E and I-A. However, about 3 years ago, Jan Klein's group at the Max-Planck-Institut für Biologie in Tübingen obtained evidence indicating that the immune responses that were supposed to be controlled by a gene encoded in the I-B region were instead mediated by an interaction of products from the I-E and I-A regions and that I-B had been an illusion. Suppressor molecules are much less likely to be artifacts in view of the large amount of evidence—albeit indirect evidence—for their existence.



The mouse MHC

The I region of the mouse MHC, which contains the genes for the class II histocompatibility molecules, is located between the gene regions (H-2K, -2D, -2L, and -2R) coding for the transplantation antigens, class I molecules that participate in such immune reactions as the recognition of viral infected cells by killer T cells. The Qa and Tla regions also code for class I molecules, but their functions are not yet known. Classic genetic mapping studies subdivided the I region as shown in the middle line of the diagram. Class II molecules consist of two protein chains, designated α and β. The genes for the two chains of the E and A molecules have now been located by molecular mapping in the positions shown. But molecular mapping has failed to find DNA that could encode the postulated I-B and I-J molecules between I-E and I-A.

The earlier mapping studies were based on the assumption that the genomes of the mice used to define the location of the I-J region differed only in the MHC. Some of the more definitive experiments were carried out with strains B10.A(3R) and B10.A(5R). The 5R strain produces a particular variant of I-J, called $I\text{-}J^k$, whereas the 3R strain does not. The I-J gene was mapped to the MHC region between I-E and I-A, because this was supposed to be the only site where the genetic makeups of the two strains differed.

What Hayes and her colleagues have now shown is that two genes are needed for production of the $I\text{-}J^k$ molecule. One of these genes is encoded outside the MHC. "This gene contributes in some way to forming the molecule called I-J," Hayes says, "but it is not sufficient. A gene in the mouse MHC is also needed." The new results suggest that the animals differ genetically outside the MHC.

In coming to this conclusion, the Hayes group first looked at the expression of $I\text{-}J^k$ by T cells from ten strains of mice, all of which have MHC genes of the k type but which have their non-MHC genes from a variety of different sources. They found that cells from two strains did not produce $I\text{-}J^k$ molecules, even though they all should have if only MHC-encoded genes are needed for its production. More than one gene appeared to be necessary.

To confirm this, the investigators performed a series of breeding experiments that essentially showed that mice bred from two nonproducer strains would make $I\text{-}J^k$ molecules as long as one parent provided the k variant MHC gene and the other parent provided the required gene from outside the MHC.

*C. E. Hayes, K. K. Klyczek, D. P. Krum, R. M. Whitcomb, D. A. Hullett, H. Cantor, *Science* **223**, 559 (1984).

Even mice bred from a 3R parent, the classic nonproducer, could make I-J^k, if the second parent contributed the non-MHC gene. Not only do the results suggest that 3R and 5R differ outside the MHC, they also indicate that 3R has the appropriate k gene in its MHC, just as 5R does. All of this is contrary to what was previously thought.

If both 3R and 5R mice have the appropriate k gene in their MHC's, then the DNA's in the region between I-E and I-A should be the same in both strains. The Hood group has found that the critical DNA segments from the two mouse strains are identical, as determined by restriction mapping—which might miss subtle differences. The investigators are determining the nucleotide sequences of the two DNA's and expect to know in a few months if they are the same.

To pin down the location of the non-MHC gene, Hayes and her colleagues turned to a series of 18 mouse strains, produced by Benjamin Taylor of the Jackson Laboratory, by crossing AKR mice with C57L mice. The Hayes group had found that AKR mice have the MHC-encoded gene needed for I-J^k molecule production but lack the non-MHC gene. The reverse is true for C57L mice. Some of the hybrids between the two strains produce I-J^k; others do not.

The distribution of several of the parental chromosomes in the hybrids has been worked out by other investigators. According to Hayes and her colleagues, hybrids that produce I-J^k consistently carry portions of chromosomes 4 and 7 from the C57L parent, but in nonproducers these chromosome segments are of the AKR type, a finding which suggests that one of the two chromosomes might carry the non-MHC gene needed for I-J production. The distribution of some of the parental chromosomes was not known, but the investigators eventually showed that chromosome 4 carries the gene, which they designated *Jt*.

What is now needed is an explanation of how two genes might collaborate to produce I-J molecules. "There is still more than one model that can account for the available data," Hayes points out. Conceivably the I-J molecule might be a modified I-E molecule, in which case the product of the *Jt* gene might be the enzyme that carries out the modification. This would be consistent with the Hood group's finding that the I-J region carries sequences coding for E_β. Moreover, the Hayes group has found that all of the I-J^k producing mice carry the k variant of the I-E genes. And Klein and his colleagues have also obtained recent evidence suggesting that I-J suppressor factors contain modified forms of I-E or I-A molecules.

Hood notes a problem with theories suggesting that the I-J molecule is a modified form of I-E, however. "If that is the case, the messenger should be there." But his group has been unable to detect messenger RNA transcripts of the DNA region between I-E and I-A in suppressor cells that make I-J.

Another, perhaps more likely, way in which the two genes might cooperate to produce I-J molecules involves the regulation of one by the other. For example, the *Jt* gene might code for the I-J structure and be controlled by the gene from the MHC. As already mentioned, the I-J molecule may be a component of T cell receptors, which can recognize a foreign antigen only in conjunction with an appropriate histocompatibility molecule—such as I-E. During development then, suppressor T cells might be selected on the basis of their having the right I-J molecule to recognize the I-E molecules carried by the immune cells with which the suppressors react.

The genes for the suppressor molecules could then appear to map to the MHC in classical genetic studies, even though they are located elsewhere, because their structures would in effect be determined by those of the corresponding histocompatibility antigens. There would have to be a way to generate the repertoire of suppressor molecules needed for the several I-E variants, but both the antibody and histocompatibility gene families provide a wealth of precedents for such generation of diversity.

This hypothesis, involving control of the *Jt* gene by one from the MHC, is not without problems of its own, however. For example, there are strains of mice that apparently do not express I-E molecules but do express I-J.

Although more work will be needed to confirm the findings of the Hayes group, the investigators have provided a valuable lead to the location of the elusive suppressor genes. Finding those genes and their products could help to solve some major problems in cellular immunology.—**JEAN L. MARX**

Additional Readings

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Through a Lens Darkly

When the first gravitational lens system was discovered, in 1979, it seemed to promise a whole new window on cosmology. Here was nature's demonstration of an effect first predicted by Sir Arthur Eddington nearly 60 years before: light from a far-distant quasar was being deflected by the gravitational field of a foreground galaxy, and was focusing into a tight little cluster of quasar images for astronomers on Earth. With luck, said the optimists, such systems might allow them to map the mass distribution in the lensing galaxy, probe the intergalactic medium, or make a more accurate determination of cosmological parameters such as the Hubble constant.

The pessimists, however, noted that the effect was both very subtle and very difficult to study, and predicted that nothing much would ever come of it. "So far the pessimists have the edge," says Irwin I. Shapiro, head of the Harvard-Smithsonian Center for Astrophysics. While the situation is far from hopeless, he adds, "the universe is an *extraordinarily* dirty laboratory."

Last month, as he reviewed the status of gravitational lens research for the American Astronomical Society (AAS),* Shapiro supported that point with a look at the five lenses that have been discovered to date. Some curiosities:

- All the quasar images come in pairs, even though the theory of gravitational optics demands an odd number of images. Perhaps the third image is just getting lost in the glare of the lensing galaxy, said Shapiro—but in all five cases? One hope for finding the lost images, if they are there at all, is with ultrahigh-resolution radio maps produced by very long baseline interferometry.
- None of the lensing galaxies lie in a straight line with their quasar images. This means that none of them has a spherically symmetric distribution of mass. Unfortunately, it also means that there is no way to deduce what the mass distributions really are because the same imagery can arise from radically different configurations. Complicating the analysis even more is the likelihood that some of the lens-

*The 163rd meeting of the American Astronomical Society, 8–11 January 1984, Las Vegas.