

Chapter 13. The I-J phenomenon

For many immunologists I-J is something that “does not exist”. In this chapter we will review some data that shows I-J to be a real phenomenon, and will see how this phenomenon can be understood in the context of the symmetrical network theory.

I-J is defined by genetics, anti-I-J antibodies, suppressor T cells and suppressor T cell factors

Immunizing an MHC^b mouse with spleen cells from an MHC^k mouse results in the production of anti-MHC^k antibodies. These can include anti-K^k, anti-D^k, anti-A^k and anti-E^k antibodies. In 1976 it was discovered that such immunizations with allogeneic¹⁸⁹ lymphocytes can cause the production of another antibody called anti-I-J^k, that had very interesting properties.¹⁹⁰ Anti-I-J^k antibodies made in an MHC^b mouse bind to antigen-specific suppressor T cell factors made by k haplotype mice, but not those made by b haplotype mice,¹⁹¹ and can be used to eliminate the suppressor T cells of the k haplotypes mouse, but not those of a b haplotype mouse.¹⁹² The converse immunization of an MHC^k mouse with MHC^b spleen cells results in the production of anti-I-J^b antibodies that bind to b haplotype T suppressor factors and b haplotype suppressor T cells, but not k haplotype suppressor T factors or b haplotype suppressor T cells. I-J was the hot topic of the time. It was expected that these antibodies would lead to the rapid elucidation of the

¹⁸⁹ Allogeneic lymphocytes are lymphocytes from an animal of the same species that has some other genes. The term allogeneic is often used in immunology more specifically to mean having different MHC molecules.

¹⁹⁰ Anti-MHC allo-antisera are sera raised by immunizing an animal with one MHC haplotype with cells from an animal of another MHC haplotype. For example the strain Balb/c has the MHC haplotype d. Its MHC molecules K, A, I and D are accordingly designated K^d, A^d, E^d and D^d, and its Ts2 suppressor T cells are recognized by anti-I-J^d antibodies.

¹⁹¹ T. Tada, M. Taniguchi and C. S. David (1976) Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody response of the mouse. IV. special subregion assignment of the gene that codes for the suppressive T-cell factor in the H-2 histocompatibility complex. *J. Exp. Med.* 144, 713-725.

¹⁹² D. B. Murphy, L. A. Herzenberg, K. Okumura, L. A. Herzenberg and H. O. McDevitt (1976) A new I sub-region (I-J) marked by a locus (Ia-4) controlling surface determinants on suppressor T lymphocytes. *J. Exp. Med.* 144, 699-712.

molecular mechanisms underlying suppression. Suppression was important for network theory, so I-J was important for network theory.

The anti-I-J class of antibodies was discovered without any knowledge about an I-J molecule, except that anti-I-J recognized an antigenic determinant on suppressor factors and on suppressor T cells. Biochemists tried hard, but frustratingly were unable to use the antibodies to purify an I-J protein from the cell surface of suppressor cells. It was only known that strains of mice that seemed to have differences in a small region of the MHC part of the genome could be used to make anti-I-J antibodies, and that these antibodies could have potent biological effects in binding to antigen-specific suppressor T cell factors and eliminating suppressor T cells.

The I-J paradox

I-J was an exciting discovery, because anti-I-J antibodies were a powerful tool for absorbing specific T cell factors and for eliminating suppressor T cells. Anti-I-J antibodies therefore seemed to be a tool that went to the heart of network regulation. The euphoria over I-J was however soon dampened. The gene or genes for the I-J determinant had been mapped by classical serological techniques¹⁹³ to the MHC locus of the mouse genome, specifically to between the genes for the class II MHC polypeptides E β and E α . (Figure 12-1). However, in 1982, using molecular genetic techniques, it was found that there is no gene at all that could encode an I-J molecule at the site in the DNA that classical immunogenetic mapping had defined as the location of the gene.^{194,195,196} This was a major embarrassment; there was no precedent for such a crass disagreement between mapping by serological techniques and molecular genetic techniques.

¹⁹³ The classical serological techniques involved making anti-I-J antibodies in several mouse strains, determining whether they are able to kill suppressor T cells in various strains, and whether they could absorb specific factors in various strains for which the various K, I-A, I-E and D genes had been mapped.

¹⁹⁴ M. Steinmetz, K. Minard, S. Horvath, J. McNicholas, J. Srelinger, C. Wake, E. Long, B. Mach and L. Hood (1982) A molecular map of the immune response region from the histocompatibility complex of the mouse. *Nature* 300, 35-42.

¹⁹⁵ M. Kronenberg, M. Steinmetz, J. Kober, E. Kraig, J. A. Kapp, C. W. Pierce, C. M. Sorensen, G. Suzuki, T. Tada and L. Hood (1983) RNA transcripts from I-J polypeptides are apparently not encoded between the I-A and I-E subregions of the murine major histocompatibility complex. *Proc. Nat. Acad. Sci. USA* 80, 5704-5708.

¹⁹⁶ J. A. Kober, E. Strauss, K. Minard and L. Hood (1986) Molecular analysis of the hotspot of recombination in the murine major histocompatibility complex. *Science* 234, 173-179.

The absence of an I-J gene or genes at the expected location put not only I-J under a cloud, but also the related phenomenon of suppression and the symmetrical network theory, that provided an explanation for suppression in terms of I-J expressing specific T cell factors. With time many immunologists found it convenient to sweep the I-J phenomenon, together with suppression and network theory, under the rug, rather than puzzle over something that didn't seem to make any sense. Most of them had not personally worked with I-J, and they were not necessarily aware of the extent of the data that show I-J to be a real phenomenon.

The mapping of I-J by classical techniques

The clearest definition of I-J is provided by two mouse strains that, in 1976, were not known to differ anywhere except in the putative I-J region of the MHC. Then they were found not to have a genetic difference even in the I-J region. The two strains are called B10.A(3R) and B10.A(5R). They were both formed as recombinants using the strains B10 and B10.A, with the recombination event being between the genes for E_{β} and E_{α} . They are commonly referred to more tersely as 3R, which has specific T cell factors expressing $I-J^b$, and 5R, which was typed as $I-J^k$. These two mouse strains were cross-immunized, and also immunized with other strains, to make anti-I-J serum antibodies, and also monoclonal anti-I-J antibodies, namely anti- $I-J^k$ and anti- $I-J^b$. There is no known difference in the genomes of 3R and 5R mice, yet 3R mice with the $I-J^b$ phenotype consistently have offspring with the $I-J^b$ phenotype, and 5R mice with the $I-J^k$ phenotype consistently have offspring with the $I-J^k$ phenotype.

The anti-I-J reagents obtained in various immunizations have consistent properties. If a mouse has the MHC^k haplotype, its suppressor cells are susceptible to killing by anti- $I-J^k$, and its specific factors can be absorbed by anti- $I-J^k$. If a mouse is MHC^b , its suppressor cells are susceptible to killing by anti- $I-J^b$ and its specific factors can be removed by anti- $I-J^b$. If a mouse is MHC^d , its suppressor cells are susceptible to killing by anti- $I-J^d$ and its specific factors can be immunoabsorbed using anti- $I-J^d$ antibodies, and so on. Such results were obtained for many different strains, so that when the I-J gene could not be found at the site in the genome where it was supposed to be, we had a robust paradox.

Towards a theory to resolve the I-J paradox

A partial resolution of the I-J paradox resulted from the work of Sumida et al.¹⁹⁷ and Uracz et al.¹⁹⁸ with chimeras, and the work of Flood et al.¹⁹⁹ with transgenic mice, which showed that the I-J phenotype of an animal depends on the MHC environment in which the T cells are selected, and not on the MHC genotype of the T cells themselves. This suggests that I-J is a V region shape or set of closely related shapes, which are selected due to the presence of MHC class II antigens. Most simply, I-J could then be either anti-MHC class II or anti-anti-MHC class II V region determinants.

Looking at I-J from the perspective of the symmetrical network theory, I-J is more likely to be anti-anti-MHC class II than anti-MHC class II. The rationale is simple, and is again based on the idea that suppressor cells have high network connectivity and helpers have low network connectivity. We argued above that anti-MHC class II cells should have low network connectivity, so they could then most simply not also be suppressors. We therefore assume, by default, that I-J bearing cells (which are primarily suppressors) are anti-anti-MHC class II.

We can then ask how anti-anti-MHC class II clones could have high network connectivity. In Figure 13-1 and Figure 13-2 we see two alternative topologies for T cells that are idiotypically connected to MHC class II antigens. In the divergent network topology picture of Figure 13-1, the anti-anti-MHC class II clones do not have a high network connectivity, and there is no reason why they should all share a particular serologically detectable shape (I-J). The alternative network focusing model of Figure 13-2 could result from a natural selection process, and seems to fit the experiments and the theory better. I-J is seen as a determinant that is selected on the basis of being complementary to the largest possible number of anti-MHC class II T cell clones. Anti-MHC class II helper T cell clones that recognize both class II MHC and I-J (I-J being anti-anti-MHC class II idiotypes) would be preferentially selected, and suppressor T cell clones that recognize as many different anti-MHC class II clones as possible would be preferentially selected at the I-J level. This part of the

¹⁹⁷ T. Sumida, T Sado, M. Kojima, K. Ono, H. Kamisaku and M. Taniguchi (1985) I-J as an idiootype of the recognition component of antigen-specific suppressor T cell factor. *Nature* 316, 738-741.

¹⁹⁸ W. Uracz, Y Asano, R. Abe and T. Tada (1985) I-J epitopes are adaptively acquired by T cells differentiated in the chimaeric condition. *Nature* 316, 741-745.

¹⁹⁹ P. M. Flood, C. Benoist, D. Mathis and D. B. Murphy (1986) Altered I-J phenotype in E_α transgenic mice. *Proc. Nat. Acad. Sci. USA* 83, 8308-8312.

Figure 13-1. A divergent network topology for clones that relate to MHC class II self antigens. Reproduced from G. W. Hoffmann et al. (1988) *The N-dimensional Network* in "Theoretical Immunology Part Two", A. S. Perelson Ed., Addison Wesley, Redwood City CA, pp. 291-319.

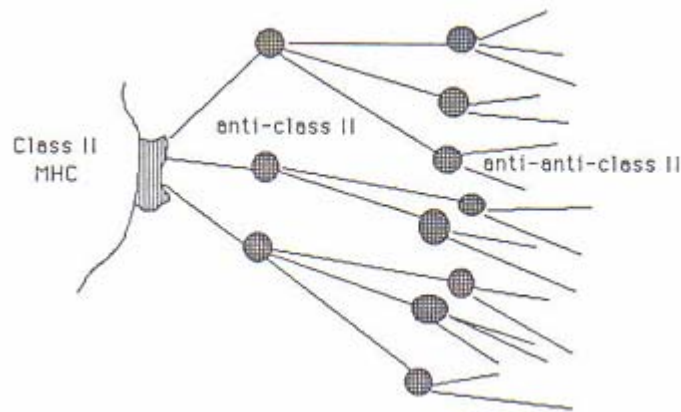
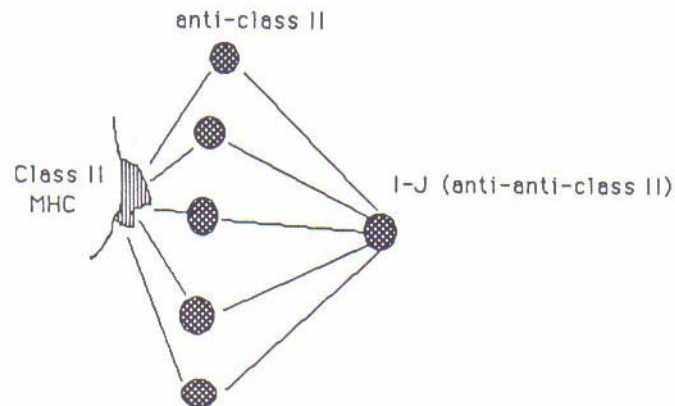


Figure 13-2. Clonal selection of T cells interacting with self MHC class II antigens and with each other leads to this "network focusing" topology. Diverse helper T cells are selected partly on the basis of affinity for MHC class II. A set of anti-anti-MHC class II suppressor T cells is selected on the basis of recognizing as many helper T cell idiotypes as possible. The anti-MHC class II helper T cells are selected to recognize the anti-anti-MHC class II suppressor T cell idiotypes in addition to MHC class II. There is co-selection of the T helper and T suppressor idiotypes, leading to the emergent selection of the idiotypes known as I-J. In the context of the helper T cell idiotypes, I-J is an image of MHC class II. Reproduced from G. W. Hoffmann et al. (1988) *The N-dimensional Network* in "Theoretical Immunology Part Two", A. S. Perelson Ed., Addison Wesley, Redwood City CA, pp. 291-319.



network has been likened to a circus tent, in which I-J is the centre-pole, and the anti-MHC class II clones are the canvas. The centre-pole is stabilized by the presence of the canvas and vice versa. This model solves some of the main parts of the I-J puzzle, as I described in references 65 and 66 in 1988.

We are thus postulating that I-J determinants are idiotopes on the V regions of suppressor T cells, that these idiotypes are selected to recognize helper T cell idiotypes, and that these in turn are selected to recognize MHC class II. I-J is then not MHC class II itself, but rather an “internal image” of MHC class II. More specifically, I-J is an image of MHC class II *from the point of view of helper T cell idiotypes*, and helper cell idiotypes are selected to recognize both MHC class II and I-J. I-J appears to map to the MHC class II region, because MHC class II indirectly selects I-J determinants. Due to symmetry in stimulatory interactions between the anti-MHC class II helper T cells and the anti-anti-MHC class II suppressor T cells, we have co-selection of helper T cells and these suppressor T cells, with I-J determinants being the emergent dominant idiotopes on the suppressor cell V regions.

If I-J is an image of MHC class II, why would anti-I-J antibodies not bind to MHC class II? The answer is that while MHC and MHC image can both have complementarity to the diverse anti-MHC V regions of helper T cells, they can nevertheless be different, as suggested in Figure 13-3.²⁰⁰

A central aspect of the I-J paradox is the question of the location in the genome of the difference between two strains that appear to differ only in I-J, namely B10.A(3R) and B10.A(5R). These two strains were thought to be genetically identical, until it was found that they have different I-J phenotypes; 3R is I-J^b and 5R is I-J^k. They have the same MHC class II molecules, and also the same MHC class I molecules. Since the only known difference is in I-J, we would like to know what the underlying genetic difference is between the two strains.

The mutual stabilization concept for anti-MHC class II clones and anti-anti-MHC class II clones leads to a novel idea about the difference between 3R and 5R. The mutual stabilization of clones with two specificity classes occurs also in the symmetrical network theory for T cells in the suppressed state, namely the co-selection of antigen-specific and antiidiotypic clones. The continued presence of the antigen is not required for the persistence of this suppressed state, because the idiootype selects for the antiidiotypic and vice versa. Analogously, it is possible that no MHC class II difference between 3R and 5R self antigens is required for a difference in the sets of T cell idiotypes to

²⁰⁰ G. W. Hoffmann and T. A. Kion (1993) Allogeneic lymphocytes as cofactors in AIDS pathogenesis and the concept of co-selection. In *New Concepts in AIDS Pathogenesis*, L. Montagnier and M.-L. Gougeon, Eds., Marcel Dekker, Inc., New York, pp. 273-290.

Figure 13-3. A schematic model of the shapes MHC, anti-MHC, MHC image and anti-MHC image. Different genes are used to produce MHC and the MHC image V regions, that are present on the suppressor T cells of the centre-pole, namely MHC genes and T cell receptor genes respectively. Hence the precise shapes of MHC proteins are different from the MHC image V regions. They are nevertheless similar from the perspective of the anti-MHC V regions. Reproduced from G. W. Hoffmann and T. A. Kion (1993) in *New Concepts in AIDS Pathogenesis*, L. Montagnier and M.-L. Gougeon, Eds., Marcel Dekker, Inc., New York, pp. 273-290.



persist from generation to generation. We would have to assume that a maternal effect plays a strong role in deciding which sets of T cell idiotypes are selected in each generation. The expression of both parental I-J phenotypes on the suppressor T cells of F1 animals (for example in a 3R x 5R mouse) indicates that there is also a paternal effect, that may be exerted via a perturbation of the maternal immune system by lymphocytes in the ejaculate.

There is experimental evidence of both paternal and maternal effects on the idiotypic repertoire. In 1980 Gorczynski and Steele reported that an MHC related phenotype that is not genetically encoded, and that is somatically acquired, can be transmitted to progeny via a paternal effect.²⁰¹ Newborn mice can be made tolerant to a foreign antigen fairly easily by injecting them at birth with the antigen, so that the immune system acquires tolerance to something that it would normally be able to respond to. This is called neonatal tolerance, and is due to the fact that the developing immune system of the newborn is very impressionable in this regard. It is even possible to induce tolerance to the very strong MHC antigens by this method. Gorczynski and Steele injected inbred mice of a strain A with large numbers of lymphocytes from cells from an F1 strain AxB, and then continued injecting the mice with the same cells, so that they became tolerant to the strain B MHC antigens. Adult tolerant strain A were then mated with normal strain A females, and 50 to 60% of the offspring were found to be specifically tolerant to in a cell mediated killing assay. There was also a significant degree of tolerance seen in 20 to 40% of the next

²⁰¹ R. M. Gorczynski and E. J. Steele (1980) *Proc. Nat. Acad. Sci. (USA)* 77, 2871-2875.

generation. Cooper-Willis et al. reported that the idiotypic repertoire can be influenced by the male parent of a mouse being immune to an antigen.²⁰² Analogous maternal effects on idiotypic repertoires have been reported by Gorczynski et al.²⁰³ and Martinez et al.²⁰⁴

The maternal effect hypothesis leads to the prediction that 3R (I-J^b) embryos transplanted into I-J^k mothers should develop into I-J^k animals, and if these animals are then inbred, their offspring, and the offspring of their offspring, should be similarly I-J^k (Figure 13-5). An analogous result is predicted for 5R embryos implanted into I-J^b mothers.²⁰⁵

It is well known that phenotype can be influenced by both genotype and environment. This has been expressed as $P = G + E$. In the case of 3R and 5R mice however, there is no known difference in either the genotype or in the environment, with the environment being simply the cages in which the mice are reared. The model to resolve the paradox in terms of co-selection of helpers and suppressors means that in addition to genome and environment, initial conditions play a role. The statement is then $P = G + E + IC$, with the initial conditions being the sole and sufficient difference between 3R and 5R.

Anti-I-J sera and anti-I-J monoclonal antibodies

Confirmation that I-J is a real phenomenon came with the demonstration that monoclonal anti-I-J antibodies can be obtained that have essentially the same specificity for suppressor T cells and suppressor T cell factors as had been shown using anti-I-J sera. Monoclonal antibodies are a more defined reagent than sera of the same specificity. A single anti-I-J monoclonal antibody species is able to eliminate suppressor T cell activity or absorb out suppressor T factors of a given haplotype.

How does one reconcile the variability of the V regions of the T cells and the T cell factors, on the one hand, with the constancy of a monoclonal antibody V region on the other? The centre-pole model describes the

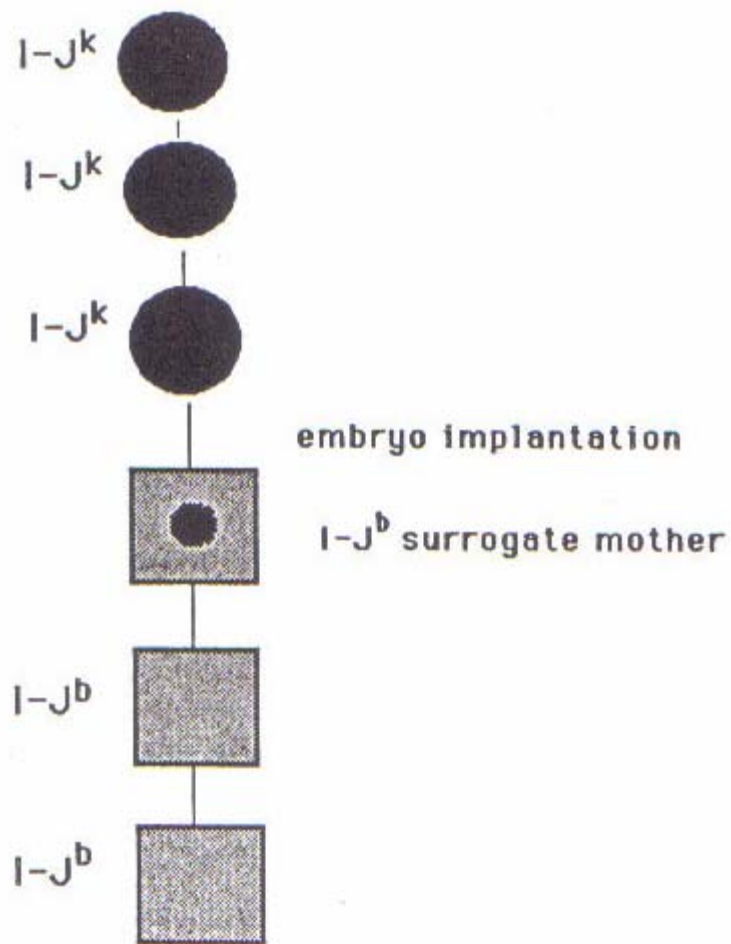
²⁰² C. A. Cooper-Willis, J. C. Olson, M. E. Brewer and G. A. Leslie (1985) Influence of paternal immunity on idiotypic expression in off-spring. *Immunogenetics* 21, 1-10.

²⁰³ R. M. Gorczynski, M. Kennedy, S. Macrae and A. Ciampi (1983) A possible maternal effect in the abnormal hyporesponsiveness to specific alloantigens in the offspring born to neonatally tolerant fathers. *J. Immunol.*, 131, 1115-1120.

²⁰⁴ C. Martinez-A., M. L. Toribio, P. A. De La Hera, P. A. Cazenave and A. Coutinho (1986) Maternal transmission of idiotypic network interactions selecting available T cell repertoires. (1986) *Eur. J. Immunol.* 16, 1445-1447.

²⁰⁵ Prediction

Figure 13-4. The hypothesis of a maternal effect on I-J phenotype predicts that if I-J^k mouse embryos are implanted into I-J^b mothers and the offspring are inbred, the resulting mice will express I-J^k. Reproduced from G. W. Hoffmann et al. (1988) in "Theoretical Immunology, Part Two", A. S. Perelson, Ed., Addison Wesley Publishing Company, Redwood City, California, 291-319.



co-selection of two shapes, one that is present on many helper cell idiotypes, and one that is present on suppressor T cell idiotypes, with the two being optimally complementary to each other. The suppressor cell idiotope is an image of the dominant MHC class II antigenic determinants, in the sense that it is complementary to helper cell idiotypes that have been selected to be complementary to the MHC class II. The part of the helper T cell V region that is used to recognize MHC class II is presumably different from the part that interacts with I-J on the suppressor cell, since otherwise conventional anti-MHC class II (anti-A, anti-E) antibodies would recognize I-J too. The co-selection of helpers and suppressors results in the selection of suppressor T cells with the I-J determinant and also the selection of helper cells that have a corresponding, complementary determinant. There is still room for specificity for the antigen in the suppressor T cells, due to the multispecificity of V regions.

I-J and breaking self tolerance: a case study

Murphy et al. found that stimulation of lymphocytes by cells of an I-J disparate strain can break T cell tolerance to self class II molecules *in vitro*.²⁰⁶ A variety of responder-stimulator combinations of cells that differed in only I-J or in I-J plus two or three other loci were cultured. The stimulators could not respond, because they were irradiated or treated with mitomycin C, which blocks cell division. The responders were free to proliferate in response to antigens on the stimulator cells. T cell lines or clones were established from the responders, which were then tested for reactivity to their own MHC class II molecules. The investigators found that in every case in which there was an I-J difference between the stimulator and responder population, self tolerance to self MHC class II was broken. Negative controls included 3R stimulated by 3R, 5R stimulated by 5R and stimulators that differed from the responders in the K region (MHC class I) and the I-A region (MHC class II). None of these controls resulted in the production of autoreactive T cell lines or clones. Evidently I-J is of relevance for self-tolerance. An interpretation in the context of Figure 13-2 is that the foreign I-J is similar to self I-J, but sufficiently different from it in that it stimulates the selection of a subset of the anti-MHC class II helper T cells. This selection constitutes a fundamental disruption of the architecture of the T cell repertoire as shown in the model. The resulting repertoire of helpers has not undergone long term selection in the context of self MHC and co-selection with corresponding suppressor T cells. In normal circumstances co-selected suppressor T cells would inhibit the emergence of helper T cell clones with reactivity directed against self MHC class II. In this

²⁰⁶ D. B. Murphy, M. C. Horowitz, R. J. Homer and P. M. Flood (1985) Genetic, serological and functional analysis of I-J molecules. *Immunol. Rev.* 83, 79-103.

case however, the perturbation by foreign I-J results in the helper T cell and suppressor T cell repertoires no longer mutually regulating and stabilizing each other.

The I-J phenomenon is based on extensive experimental observations

There is no credence to the various ways in which some immunologists have attempted to sweep the I-J phenomenon under the rug. For example, the suggestion that "I-J is an artifact" is not a scientific explanation of anything. Nor does the observation that "we haven't heard much about I-J lately" discredit I-J. The I-J phenomenon is based on a lot of data that looks convincing, and there is no reason to assume that old data is worse than new data. Recently published data ignores I-J, it does not debunk it. The genetic data mapping of I-J is extensive. It was mapped with considerable redundancy by Murphy et al. and Tada et al. using many strains of mice. Subsequent work has shown that I-J consistently maps to the MHC in experiments using many different strains, including MHC congenic strains.^{207,208} I-J determinants are consistently found on suppressor T cells and suppressor T cell factors. Monoclonal antibodies have been made that are anti-I-J, and these give results consistent with those obtained using anti-I-J sera. If I-J were an artifact, we would not have so much self-consistent I-J phenomenology.

I have emphasized that focusing on paradoxes is a good way to progress in the formulation and development of theory. The I-J associated paradox emerged in 1982, and an interpretation in terms of the symmetrical network theory was published in 1988. In the 1990s I-J became an unfashionable topic, in spite of its importance as a paradox, and then as a resolved paradox. **Figure 13-6** is a graph of the number of papers published from 1976 to 1991 that have I-J in the title. The subject was abandoned without anyone making an even half convincing case that the original data establishing the phenomenon was flawed, or that the theory explaining it was flawed. I-J is currently an important, largely forgotten story.

²⁰⁷ MHC congenics are pairs of strains that have the same background genes and different MHC haplotypes.

²⁰⁸ A report that one H-2^k strain (AKR/J) did not express I-J^k was quickly refuted. The claim was made in C. E. Hayes, K. K. Klyczek, D. P. Krum, R. M. Whitcomb, D. A. Hullet and H. Cantor (1984) The chromosome 4 Jt gene controls murine T cell surface I-J expression. *Science* 223, 559-563. The refutation is in P. M. Flood and D. B. Murphy (1985) The putative I-J^{k-} strain AKR/J synthesizes I-J^{k+} molecules associated with suppressor factors: Implications for Jt control of I-J expression. *J. Mol. Cell. Immunol.*, 2, 95-103. The level of expression of I-J^k on lymphocyte surfaces in AKR/J mice is however much lower than in the prototypical I-J^k strain, namely B10.A(5R).

I-J is an unfinished story, with more complexity

The phenomenology associated with I-J is extensive, and much of it was only beginning to be worked out when the I-J paradox came along and many researchers lost interest. (This was exactly the wrong response; the paradox should have added to the level of interest in I-J, rather than decreasing it.) The model I have presented above accounts for basic, well established aspects of I-J (including the genetic paradox), but much more has been published on I-J that is less well known. For example, there is evidence of multiple I-J determinants. I-J has been detected on multiple suppressor T cell populations and also on other cell types including certain helper T cells and contrasuppressor cells. There is variability in some of the finer details obtained in different experimental systems, but there is also much common ground. I will have more to say about I-J in the next chapter and especially in chapter 17, where I describe how the theme of co-selection can be extended to encompass much of the additional data.