T Cells Scrutinized at Rüdesheim Meeting

T cell behavior and how it is affected by the thymus were discussed. Not all of the confusion was resolved

Cellular immunologists gathered recently in Rüdesheim am Rhein, Germany, to assess the state of knowledge about the thymus gland and its influence on the activities of T lymphocytes.* These cells, which mature in the thymus and play a central role in immune responses, have two major functions. They can directly kill certain types of undesirable targets, such as virus-infected cells. And they can regulate other immune cells, including B lymphocytes, the antibody producers. To perform these jobs, T cells must interact directly with their various targets.

Three intertwined themes dominated the discussions at Rüdesheim. Two of these were the nature of the "restriction" displayed by T cells in their activities and the role of the thymus in generating the restriction patterns of the cells. The third theme, underlying the other two, was the nature of the ever elusive T cell receptor, the cell component—or components—by which T lymphocytes recognize and interact with the appropriate partners. As Alfred Singer of the National Cancer Institute (NCI) puts it, "The receptor is at the heart of all discussion about the T cell."

Beginning in the early 1970's investigators showed that an immune cell can interact with its partner only when both cells have in common certain molecules that are located on the cell surfaces and coded for by genes present in the major histocompatibility complex (MHC). Immune cells that can attack or regulate only cells of the same histocompatibility type are said to be self-restricted.

Until the discovery of restriction, the only known role for the histocompatibility antigens had been that of triggering the rejection of transplanted organs by the recipient's immune system, a highly artificial situation. But the existence of restriction made it clear that the antigens are intimately involved in the ordinary activities of immune cells. MHC restriction became, says Peter Doherty of the Wistar Institute, "the touchstone of all research in cellular immunology."

The discovery bore directly on the T cell receptor issue. Normal interactions of self-restricted immune cells differ in a crucial aspect from transplant rejection. The attack of immune cells on foreign tissue is triggered by the foreign histocompatibility antigen alone. In a normal immune reaction, one in which a killer T cell attacks a virus-infected cell, for example, the killer must recognize two entities, self in the guise of the appropriate histocompatibility antigen, plus a foreign antigen, such as a viral component on the surface of an infected cell.

Consequently, the discovery of self-restriction gave rise to two competing theories about the T cell receptor. The dual recognition theory, which was proposed by Baruj Benacerraf of Harvard Medical School and David Katz, who is now at the Scripps Clinic and Research Institute, holds that recognition of antigens by T cells requires two separate receptors, one for the foreign antigen and the other for the histocompatibility antigen. In contrast, the altered-self theory, as suggested by Doherty and Rolf Zinkernagel, who is currently at the University of Zurich, holds that there is one receptor that recognizes the self-antigen only when it has been modified by interacting with the foreign antigen.

A second key discovery, made about 3 or 4 years ago, concerns the role played by the thymus in generating self-restriction and the T cell repertoire in general. At that time, research by Zinkernagel and Michael Bevan of the Massachusetts Institute of Technology (MIT) indicated that self-restriction is not an inherent property of T cells but is learned during their sojourn in the thymus.

Zinkernagel then began to favor the dual recognition view. In an interview with *Science* early in 1979 he said, "Now it looks as if restriction is determined independently of antigen during the T cell maturation process. It supports the theory that there are two receptors, but it is not unequivocal proof."

Bevan continued to support the altered-self theory, however. "Self-restriction by the thymus is more easily explained by the two-receptor model. But I am a one-receptor person and still believe in self-restriction." Bevan postulates, in accordance with a proposal by Niels Jerne of the Basel Institute of Immunology, that when T cells encounter the cells of the thymic epithelium, those having receptors that recognize the self antigens of the thymic cells are stimulated to divide. As they divide, mutations may occur to produce slight alterations in receptor structure. Eventually, individual members of the resulting population will be able to recognize the appropriate self-antigen as modified by one or another foreign antigen.

Although there was disagreement 3 or 4 years ago, as there is today, about the T cell receptor, the role of the thymus as the teacher of self-restriction seemed firm. But more recent experiments have sometimes produced results not compatible with that view. "During the past 3 years," says Singer, "we began seeing a variety of discrepancies with what the thymic hypothesis predicts."

The stated goal of the Rüdesheim meeting was to sort out the disparate results to find points of agreement about the influence of the thymus on restriction. But speaker after speaker presented results that often seemed irreconcilable. "It was difficult to tell what was happening from that meeting," concedes Irving Weissman of Stanford University Medical Center. "I work in the field and I am confused. Reputable people get contradictory results."

Despite the considerable surface confusion, however, private discussions revealed substantial agreement that while the influence of the thymus on restriction is not absolute and probably not unique, it is still very important. Still not clear is the nature of the T cell receptor. Although two sets of experiments strongly supported the altered-self theory, the supporters of dual recognition have ammunition of their own and are not ready to surrender.

The thymic hypothesis predicts that T cells will be restricted to recognizing target or partner cells of the same histocompatibility type as the thymus in which the T cells matured. Normally, of course, the T and thymic cells of a particular animal have the same MHC antigens. To study thymic influences on restriction, investigators often use ex-

*The workshop, "The role of the thymus in the generation of the T cell repertoire," was organized by Herman Wagner, Martin Röllinghof, and Klaus Pfizenmaier of the Johannes-Gutenberg-Universität Mainz and was held on 16 to 19 September.

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perimental models, such as bone marrow chimeras, in which the thymus gland and the differentiating T cells have different histocompatibility makeups.

To construct a bone marrow chimera, mice of a particular histocompatibility composition (designated as A in the usual shorthand) are first lethally irradiated to kill their immune cells and immune cell precursors. Then they receive a transplant of immune cell precursors, such as bone marrow that has been treated to remove contaminating mature T cells, from mice of a different MHC type (called B). Because thymic epithelial cells are more resistant to radiation than are immune cells, they can still foster T lymphocyte maturation.

In the early experiments of Zinkernagel and Bevan, the transplanted material was taken from mice that were produced by mating type A with type B animals. Normally, killer T cells from hybrids like these should be able to respond to a foreign antigen (denoted by X) present on target cells of either the A or the B type. However, Zinkernagel and Bevan showed that when killer cell precursors from A × B hybrids mature in A animals they recognize X only on type A cells, or at least with a very strong preference for type A cells. When they mature in B animals they see X in conjunction with B histocompatibility antigens.

That the thymus was the site of these alterations in responsiveness was shown by a more complicated version of these experiments in which the A × B mice were thymectomized as well as lethally irradiated. The mice were then given transplants of an irradiated A or B thymus and A × B bone marrow. In this case the T cells were restricted to the MHC type of the transplanted thymus.

Other investigators have failed to find thymic influences on self-restriction in bone marrow chimeras, however. For example, Katz showed that the thymus did not restrict partner cell preferences displayed by helper T cells differentiating in chimeras.

Helper T cells stimulate antibody production by B cells in response to certain antigens. This interaction is restricted, as Katz and Benacerraf showed in the early 1970's. The genes for the restricting antigens are located in the I (immune response) region of the MHC, whereas those for the transplantation antigens involved in killer cell interactions are in the K and D regions.

Nevertheless, the differences between Katz's results and those of Zinkernagel and Bevan probably cannot be attributed to anything as simple as a class difference between killers and helpers. Other investigators, including Singer, Ronald Schwartz of the National Institute of Allergy and Infectious Diseases, and Jonathan Sprent of the University of Pennsylvania School of Medicine, have found that the thymus clearly imposes restriction on helper and other regulatory T cells. And, just to bring the confusion full circle, researchers have sometimes found that killer T cell responses are not restricted to thymic MHC antigens in bone marrow chimeras.

Several participants at the Rüdesheim meeting suggested that many of the contradictory results obtained with bone marrow chimeras might have been caused by the complexity of the experimental system. As Herman Wagner of Johannes-Gutenberg-Universität Mainz points out "A great deal of information can be achieved by using this model, but the animal has a lot of variables." The complexity of the model is compounded by the variability in the experimental designs: no two chimera experiments seem to be exactly the same.

In any event, two of the variables that have emerged as important are the amount of radiation the animals receive before they are given the bone marrow transplants and the time after transplantation when the T cells are assayed for their particular activity.

The amount of radiation is important because, as Weissman says, "Any dose of radiation that we give that allows the animal to recover won't kill all the mature T cells." These cells, which had already matured in the host thymus, could certainly confound the results obtained with bone marrow chimeras that also received thymic transplants.

Doherty, and also Sue Sharrow and her colleagues at the National Institutes of Health, showed that the first cells to repopulate the thymuses of some bone marrow chimeras are, in fact, of host origin. This occurs about 2 weeks after irradiation and reconstitution with bone marrow. In another 1 to 2 weeks the host cells disappear and cells of bone marrow origin take their place.

Schwartz, with Dan Longo of NCI, suggested another way in which changing cell populations in the thymus might alter restriction specificities, at least for helper cells. One of Longo's findings, which Schwartz described at Rüdesheim, is that the amount of radiation influences the speed with which bone marrow cells move into the irradiated thymuses of chimeras, with larger doses favoring faster movement.

The two investigators previously reported that restriction of helper cells appears to be determined not by the cells of the thymic epithelium but by the presence in the thymus of a type of bone marrow cell with which helper cells are known to interact. If both these results are correct, investigators who give higher doses of radiation might not see thymic influences on restriction. Singer concludes, "The differences are due to the way the different groups do their experiments, especially the radiation they give." Not everyone agrees that it is the bone marrow cells that determine restriction in the thymus, however. Evidence from Zinkernagel's and Bevan's laboratories suggests that the restriction specificities of cytotoxic T cells might be determined by thymic epithelial cells.

"The receptor is at the heart of all discussion about the T cell."

Experiments with nude mice, which are not supposed to have a functional thymus but which do have a rudimentary gland, also produced results that are inconsistent with the thymic hypothesis. When Berenice Kindred of the Max-Planck-Institut für Biologie in Tübingen transplanted thymuses of one histocompatibility type into nude mice of another type, she often found that the animals' T cells were restricted not to the thymic type but to that of the mouse.

Much of the discussion at Rüdesheim about the results with nude mice had to do with whether this animal is a good model of the "prethymic repertoire." The answer was that it probably is not.

The T cells of nude mice were supposed to be different from those of normal mice by virtue of never having matured in a thymus. They should thus represent a sort of ground state (the prethymic repertoire, in immunologists' jargon). By comparing them with T cells that had matured in the thymus, researchers might determine what the gland is doing.

More recently, research done by Thomas Hünig of the University of Würzburg while he was a postdoctoral fellow in Bevan's laboratory, by Jean-Charles Cerottini of the Ludwig Institute for Cancer Research in Lausanne, and by Wagner suggests that nude mice, especially aged ones, have at least a few T

cells that behave like the T cells of normal mice. They may not be detectable in the animals, but they are seen when stimulated by appropriate growth factors in culture. Wagner explains, "You can get clones that are by all criteria functional T cells in vitro." In short, if nude mice have T cells that have already undergone some kind of maturation before the animals receive a thymus transplant, their T cells could display restriction characteristic of the host and not of the transplant.

The existence of these T cells in nude mice poses another question, of course: where do they come from? Although nude mice have a rudimentary thymus, there is little evidence that it fosters the maturation. The other obvious possibility is that the T cells mature by a pathway not requiring a working thymus.

Such a possibility was also suggested by chimera results obtained by Singer and Doherty. They found that cells from the thymus are more stringently restricted to recognizing thymic antigen than cells from the periphery.

In a related development, Richard Miller of the Ontario Cancer Institute, Toronto, found that when immature T cells from the spleen are cultured under appropriate conditions, a single cytotoxic precursor cell can have restricted progeny with different specificities. This result also favors the idea that the periphery can influence T cell specificity.

Such influences would not be surprising to some researchers. Katz, for example, has long favored this view. He also thinks that the emphasis on the thymus has been overdone. He explains, "The central issue is that lymphocytes adaptively differentiate and learn their proper partners. Where it happens is an issue of secondary importance."

Finally, results from a third set of experiments, this time with T cells from normal mice, raised questions about the role of the thymus. These results, obtained in the laboratories of Wagner and of Doherty, showed that normal mice, in addition to having self-restricted T cells, often had allorestricted (other restricted) cells that were capable of recognizing a foreign antigen when it was presented with histocompatibility antigen of some type other than the animal's own.

To do this type of experiment, the researcher must first remove from the T cell population those members that would launch a graft rejection attack on cells bearing the nonself MHC antigen. Once this is done, those remaining can be tested for the presence of allorestricted cells. Usually, these were found to be present, although in much lower frequencies than self-restricted cells.

These results imply, according to the Wagner group, that the thymus does not dictate restriction specificities absolutely and that restriction can be elicited in the periphery. As mentioned previously, others have made similar suggestions. And even in the earliest experiments on the role of the thymus, there were sometimes indications that restriction specificities it imparted were not absolute.

Wherever the specificities are imparted, their nonabsolute character implies that there might be cross-reactivity in the T cell repertoire; that is, a T cell that responds to one combination of foreign and histocompatibility antigen might also respond to a different combination. The existence of cross-reactivity would bear directly on the T cell receptor question as it would make dual recognition theories harder to defend.

In experiments done, as most have been, with mixed populations of cells, it is not possible to tell whether a particular individual is cross-reactive. Recently, however, investigators have learned how to produce T cell clones, in which all the cells of a population are derived from a single cell and should be identical. When they look for cross-reactivity in the clones they often find it, as two groups reported in Rüdesheim.

Schwartz described clones of T cells, possibly helpers, that respond both to foreign antigen X plus histocompatibility antigen A and to histocompatibility antigen B alone. He says, "There are a lot, approximately 20 percent, of antigen-specific clones that have alloreactivity."

In addition, Hünig and Bevan found a clone of cytotoxic T cells that can respond to target cells of two different specificities. They recognize both A plus conventional antigen X and B plus conventional antigen Y. As the investigators point out, this result "is quite inconsistent with dual recognition models which postulate independent recognition of self H-2 [histocompatibility] antigen and foreign X by separate receptor sites." The receptor is apparently recognizing not the individual parts but the sum thereof.

Even Katz, who has been described as the "archetypal supporter of dual recognition theories," concedes, "These clones certainly can constitute a thorn in the side of dual recognition theories." But then he adds a note of caution, "If there are no problems with the experiments. They require further scrutiny."

Investigators have been taking these indirect means to pin down the T cell receptor because so far they have not been able to isolate and study it directly. As Melvin Cohn of the Salk Institute explains, "We have no chemistry for the T cell receptor. We have to derive its structure from the physiology."

The T cell receptor is a membrane structure, and isolating membrane components that retain their activity is often very difficult. In an attempt to circumvent this problem, a number of investigators have been looking at molecules (usually called factors) from the fluid in which cultured T cells grow.

The investigators cite the example of the B cell receptor for antigen, which is known to be membrane-bound antibody with a structure similar to that of the soluble antibody secreted by the cells. Moreover, investigators, including Klaus Eichmann of the Max-Planck Institut für Immunbiologie in Stübweg, Klaus Rajewski of the Institut für Genetik in Cologne, Hans Wigzell of Uppsala University, and Hans Binz of the University of Zurich, have provided indirect evidence for the expression of genes coding for the variable regions of antibody heavy chains in T cell receptors.

Among those presenting their data on "factors" at Rüdesheim were Harvey Cantor of Harvard Medical School and Richard Gershon of Yale University School of Medicine. Cantor produced a clone of helper T cells that are activated by a foreign antigen, insulin, when it is presented on cells with the appropriate MHC antigen. "The activation is very specific," Cantor says. "The clone is activated by beef insulin and MHC product, but not by pig insulin and MHC product." The two insulins differ by only two amino acids.

Cantor found several polypeptides that were synthesized by the clone and secreted into the culture fluid. They bound to insulin with the same specificity as that displayed by the activation reaction. At least two of the proteins participate in this highly specific binding.

"The binding specificity of the secreted peptides accounts for the specificity of activation of the mother clone," Cantor says, "but the significant thing is that the helper cell can't bind free insulin. It is activated only when the antigen is presented on a cell with the right Ia [MHC] molecule. Our working hypothesis is that the two peptides are not associated on the cell membrane but are brought together by the Ia antigen complex." If the hypothesis is correct, activation would require two separate recognition events, one of histocompatibility product and one of the specific antigen.

Gershon and his colleagues isolated two proteins that stimulate the activity of T cells that suppress antibody production by B cells. Both proteins must act

together to produce the effect. One of them binds antigen and the other recognizes self, although in this case the restriction is linked to immunoglobulin genes. According to Gershon, these results must be explained in terms of dual recognition if the two molecules act on the same cell. They cannot be interpreted in terms of altered-self theories because the self-recognizing molecule will not work by itself.

Gershon sums up the current status of T cell receptor theories in the following words. "People came to Rüdesheim with certain commitments to possible theories. Extremely interesting data were presented by each to establish his view. As clever as the experiments were, people with the opposite view could think of more clever explanations for them. The tie breaker has not yet appeared in this particular match."

The ultimate tie breaker will be the isolation of the receptor molecules themselves and the identification of the genes coding for them. Because of the evidence that heavy chain variable genes code for a portion of the receptor, researchers are looking for gene rearrangements during the development of T cells analogous to those known to occur during the differentiation of B cells. The rearrangements might serve as a guide to the receptor genes. So far, as reported by Susumu Tonegawa, who recently moved from the Basel Institute of Immunology to MIT. "We don't know anything about the structure of the genes coding for the T cell receptor." His laboratory and others have shown that J_H gene segments, which code for the region that connects the variable and D regions with the constant region of the heavy chain, are not used to code for the receptor. That does not rule out participation of the V region, however, and Tonegawa is still looking for a rearrangement involving this gene segment.

Although there may still be uncertainties about the role of the thymus and certainly about the nature of the T cell receptor, researchers have come a long way in the past decade toward understanding the working of histocompatibility antigens. Katz says, "We have learned three things indisputably. Cells are restricted to recognizing self; that self-recognition is a crucial aspect of their function; and they have sufficient plasticity to adapt to the environment where they differentiate." One day there may even be agreement about the T cell receptor.—JEAN L. MARX

Additional Reading

1. Overview of the major histocompatibility complex: B. Benacerraf, *Science* **212**, 1229 (1981).
2. Experiments with chimeras: D. L. Longo, L. A. Matis, R. H. Schwartz, *Crit. Rev. Immunol.* **2**, 83 (1981).
3. One-receptor view of T cell behavior: P. Matzinger, *Nature (London)* **292**, 497 (1981).
4. Two-receptor view: D. H. Katz, *Adv. Immunol.* **29**, 137 (1980).
5. Short review on "Thymic education": M. J. Bevan, *Immunol. Today*, in press.

Impact Looks Real, the Catastrophe Smaller

Diverse specialists now agree that the evidence for a huge asteroid (or comet) impact is impressive, but they have scaled down its effects

The notion may have seemed fanciful at first, the idea that an asteroid the size of Manhattan might have rammed into Earth, darkened the skies for 3 years with the dust it kicked up, and killed off all manner of plants and animals including the dinosaurs. But the kernel of evidence that in early 1980 inspired this particular version of mass extinction received early support from some scientists (*Science*, 31 October 1980, p. 514). At a meeting* last month called to take a serious look at the proposal, geochemists, as well as paleontologists, geologists, and physicists, agreed that there really do seem to be chemical traces of an impact 65 million years ago.

The theory also received a boost at the meeting when experts resolved several problems concerning its physical plausibility. The most pivotal change was forced when, late on the first morning of the meeting, Brian Toon of the National Aeronautics and Space Administration's Ames Research Center at Mountain View, California, shrank the ominous 3-year period of darkness down to a more comfortable 3 months or so. That pleased the terrestrial paleontologists, who had been insisting all along that the proposed catastrophe was much too severe. Such a scenario simply did not jibe with their fossil record of limited, though extensive, extinctions at the boundary between the Cretaceous and Tertiary periods 65 million years ago.

The problem has been with the dust. The originators of this particular impact scenario,† Luis Alvarez, Frank Asaro, and Helen Michel of the University of California's Lawrence Berkeley Laboratory and Walter Alvarez at the University of California at Berkeley, modeled their "great darkness" on the atmospheric effects of the eruption of Krakatau, as reported in an 1888 publication. Toon pointed out that the Alvarez group had incorrectly assumed that the dust stayed up for the full 3 years that dramatic sunsets had been seen around the world. Rather, the dust probably fell out in about 3 months, Toon said, leaving the volcano's sulfurous gases to form a persistent haze in the stratosphere.

Even if the amount of dust were to be increased a thousandfold or more, it would not help, Toon noted. Particles as small as 0.5 micrometer could last 1 to 2 years if not for their inevitable tendency to stick to one another, form larger particles, and fall out at the faster rates typical of larger particles. According to calculations by Toon and James Pollack of Ames, the longest that the "darkness at noon" could have lasted was 4 to 6 months.

After the drastic downward revision of the duration of the darkness, paleontologists could finally make some sense of the impact hypothesis. The Alvarez group had postulated 3 years of darkness, cessation of photosynthesis, and the complete collapse of food chains on land and in the sea from the bottom up. Survivors would have included plants regenerated from long-lived seeds, spores, and root systems, and the small animals that could have eaten insects and decaying vegetation.

*Conference on Large Body Impacts and Terrestrial Evolution: Geological, Climatological, and Biological Implications, 19 to 22 October 1981 at Snowbird, Utah; sponsored by the Lunar and Planetary Institute and the National Academy of Sciences. Meeting abstracts may be obtained from Library/Information Center, LPI, 3303 NASA Road 1, Houston, Texas 77058. Enclose a check for $3 (U.S. domestic and foreign surface mail) or $5 (foreign airmail).

†W. M. Napier and S. V. M. Clube of the Royal Observatory, Edinburgh, have also suggested that the blockage of sunlight by the dust from a large impact could produce mass extinctions [*Nature (London)* **282**, 456 (1979)].

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