The T Cell Receptor—At Hand at Last

Several investigators have prepared monoclonal antibodies that identify molecules with the predicted characteristics of the T cell receptor

Few issues in science have proved as difficult to resolve and as subject to contention as the nature of the T cell receptor, the surface structure by which T cells recognize specific antigens. This recognition is essential for activation of the cells, which have two major functions in the immune system. They kill cells that appear foreign, such as cells that have been infected with viruses and carry viral antigens. And they regulate other immune responses, including antibody production by B cells.

As long as the identity of the T cell receptor was unknown, a molecular understanding of the cellular interactions underlying these activities was not possible. Now at least four groups of investigators have produced monoclonal antibodies that recognize T cell surface proteins with the predicted characteristics of the receptor. The molecules have not yet been definitely proved to be the receptor; however, the results from the different laboratories agree very closely. "All the data taken collectively strongly suggest that the T cell receptor has been defined," says Ellis Reinherz of the Dana-Farber Cancer Institute and Harvard Medical School.

All of the groups used similar approaches. They prepared monoclonal antibodies against lines of cloned T cells and looked for those antibodies that reacted only with the cells used to elicit the antibody production in the first place.

James Allison and his colleagues at the University of Texas System Cancer Center in Smithville published the first report in which a monoclonal antibody was used to identify a candidate for the T cell receptor. The discovery came, as many do, by accident. The investigators were attempting to prepare monoclonal antibodies against surface antigens that might be characteristic of particular tumors, in this case a T cell lymphoma of the mouse. Such monoclonal antibodies might eventually be used to diagnose or treat cancers.

To prepare monoclonal antibodies, animals are first immunized with the desired antigen. Antibody-producing spleen cells from the animals are then fused with myeloma cells to produce clones of hybrid cells called hybridomas, which make the antibodies.

When Allison and his colleagues immunized mice with a line of cloned T lymphoma cells, they obtained a hybridoma that produced a monoclonal antibody that reacted only with the immunizing cell line and not with other lymphoma lines or with other types of normal lymphoid cells. The antigen with which the antibody reacted might have been the desired tumor-associated antigen, or it might, as further work by the Allison group indicated, be even more interesting than that.

They found that the target antigen consisted of two polypeptide chains linked together by disulfide bonds. The chains had molecular weights of about 39,000 and 41,000.

> The results from the different laboratories agree very closely.

The researchers went on to show that similar molecules could be identified in preparations of cell surface molecules from other T cell lymphomas, and from normal T cells and thymus cells. (The thymus is the organ where T cells mature.) They were not present in comparable material from B cells or spleen cells, however. Allison and his colleagues suggested that the antigen identified by their monoclonal antibody is specific to the original T cell clone used to prepare the antibody, but that variants of the antigen might be found on all T cells.

That is one of the required characteristics of the T cell receptor. The molecule should be found on all immunocompetent T cells, but as members of different clones recognize different antigens, the fine structure should vary slightly from clone to clone.

Allison and his colleagues suggested that the molecule they identified could be a candidate for the T cell receptor. They could not prove this, however. The T lymphoma line they had used did not perform any T cell functions, and they could not tell whether the monoclonal antibody would block T cell activation, a sine qua non if it was in fact reacting with the receptor.

Because the cell lines used by the other three groups to elicit monoclonal antibody production do carry out T cell activities, these studies provide direct evidence that the antibodies are identifying the T cell receptor. Reinherz and Stefan Meuer, also of the Dana-Farber Cancer Institute, who have been working with human T cells, obtained monoclonal antibodies to two clones of normal cytotoxic T cells. The two lines were obtained from the same individual, but have different antigen specificities. One clone, which is designated $CT8_{III}$, kills target cells bearing a particular class I histocompatibility antigen. The second line, which is designated $CT4_{II}$, recognizes a class II, rather than a class I, histocompatibility antigen. The monoclonal antibodies raised against these cell lines blocked both cell-killing and proliferation in response to the antigens. They were also very specific. Antibody against $CT4_{II}$ did not affect the responses of $CT8_{III}$ and the two antibodies obtained against $CT8_{III}$ did not block the responses of $CT4_{II}$. Nor did the antibodies react with any of the other cell lines tested, including 80 additional T cell clones from the same individual.

The T cell surface antigens with which the monoclonal antibodies react have properties similar to the molecule identified on the lymphoma cells by the Allison group. "The structures that the antibodies were defining," Reinherz says, "were disulfide-linked heterodimers [molecules composed of two different polypeptide chains]. The molecular weights of the two chains turned out to be 43,000 and 49,000."

Another indication that the monoclonal antibodies are identifying the T cell receptor comes from studies in which the antibodies are bound to a solid support such as Sepharose beads. The Harvard workers find that under these conditions the antibodies can mimic antigen by stimulating proliferation and interleukin-2 release by the corresponding clones

instead of inhibiting T cell activation by antigen as the soluble antibodies do. (Interleukin-2 is released during T cell activation and stimulates the proliferation of T cells.)

The bound antibodies have a stimulatory effect, apparently because they permit cross-linking among receptors similar to that occurring when an activating antigen binds. The soluble antibodies are inhibitory because their attachment to receptor molecules prevents antigen binding but does not produce cross-linking. As expected, the soluble antibodies block stimulation by the corresponding bound antibodies, Reinherz says.

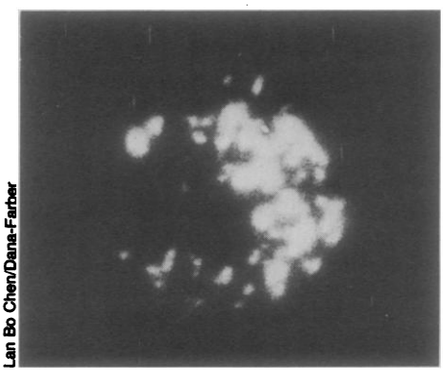
Meanwhile, John Kappler and Philippa Marrack of the National Jewish Hospital and Research Center and the University of Colorado Health Sciences Center in Denver had been developing a slightly different approach to the problem of obtaining enough T cells to study. Instead of using clones of normal cells, they fused normal cells with T tumor cells to make functional T-T hybridomas. As Kappler and Marrack showed, the appropriate antigens stimulate production by the hybridomas of interleukin-2.

Even with an abundant source of functional cells in hand, the production of the desired monoclonal antibodies did not come easily. Kappler and Marrack first used the T-T hybridomas to immunize rats in their search for antibody-producing hybridomas. Kappler says that they screened "probably 5000" hybridomas for monoclonal antibodies to the receptor and that they "didn't find any."

Then, with Kathryn Haskins and Ralph Kubo, also of the National Jewish Hospital, they switched to immunizing mice. Now the Denver workers have three monoclonal antibodies that specifically block antigen-induced release of interleukin-2 by the clone used for the immunization. The antibodies bind to T cell molecules, again heterodimers composed of two different chains which are linked by disulfide bonds and have molecular weights in the range of 40,000 to 45,000.

Finally, Lawrence Samelson and Ronald Schwartz of the National Institute of Allergy and Infectious Diseases also used cloned T cell hybrids to elicit monoclonal antibody production. They obtained two antibodies specific for the same clone; these inhibit the response to antigen of that clone and identify the usual heterodimer.

The specificity of the monoclonal antibodies for a given T cell or T-T hybridoma clone is one indication that the target molecules vary from clone to clone as expected for the T cell receptor. Another



Lan Bo Chen/Dana-Farber

T cell stained for T3

Fluorescence micrograph shows T3, a protein which may be an invariant part of the T cell receptor. Staining leads to aggregation of the molecules.

is provided by the chemical analysis of the T cell molecules themselves.

For example, Oreste Acuto of the Harvard group is comparing the proteins from different clones and finds that they are chemically distinct. "The proteins vary from clone to clone," Reinherz explains, "and this peptide variability is likely to be responsible for the antigenic specificity." Both chains of the heterodimer are variable, the Harvard workers find, although there may be more variability in the smaller chain. The result implies that the receptor molecule is encoded by two separate genes.

Kappler and Marrack have compared peptide maps of T cell molecules from two of their own clones and from that of the Allison clone, which comes from a different mouse strain. "Much to our surprise they are very different," Kappler says. "When we compare the Allison line with ours, we can't find a peptide in common. Even when we compare our own two, of the same strain, they are very different."

Kappler and Marrack were apparently looking at the more exposed and variable region of the T cell molecule. The general structural plan of the T cell receptor for antigen is expected to resemble that of the B cell receptor, which is membrane-bound immunoglobulin (antibody). Immunoglobulins have a constant (framework) region, which is close to the membrane, and a variable (antigen-binding) region, which is outside and exposed to the surroundings.

The Allison group found evidence of both constant and variable regions when they compared peptide maps of T cell receptor candidates from different clones. Allison says, "We saw six common fragments, plus quite a number that were different from cell to cell." Allison and his colleagues have also prepared an antiserum that recognizes the framework region, which may help in isolating additional T cell receptor molecules.

The next step is the determination of the partial amino acid sequences of the receptor proteins. As Kappler puts it, "We are all racing to get partial amino acid sequences because that is the ticket to the genetic material." The nucleotide sequences of the corresponding gene segments can be deduced from the amino acid sequences. The nucleotides can be synthesized and used as probes to detect and isolate the genes. Then it will be possible to map the chromosomal position of the genes and tackle such questions as the origin of diversity in the T cell receptor and the nature of the relationship between the genes coding for T cell receptor molecules and those coding for immunoglobulins.

Many immunologists had thought that T cell receptors might incorporate the same variable segments as those used for making immunoglobulins. Both have to recognize an essentially unlimited range of antigens. Kappler points out, "It seemed impossible to ask nature to come up with a sophisticated recognition system like that twice."

The evidence for immunoglobulin variable regions in T cell receptors has been equivocal at best, however. But once T cell receptor genes are cloned, they can be compared directly with those for immunoglobulins. Most investigators expect that the genes for the T cell receptor will constitute a separate family, although they may be related to the immunoglobulin genes, perhaps as the genes for histocompatibility antigens are. Samelson and Schwartz have evidence that there may be disulfide bonds within the individual chains of the T cell receptor molecules in addition to those between chains. Immunoglobulins have both types of disulfide bonds.

Identification of the T cell receptor molecules may also help resolve the long-standing debate about the manner in which the cells recognize their targets. Many T cells are said to be "restricted." They are not activated by foreign antigen alone, but only when the antigen is presented on the surface of another cell of the same histocompatibility type.

The discovery of restriction gave rise to two competing theories about the nature of the T cell receptor. The dual recognition theory holds that the receptor has separate components for recognizing the foreign and histocompatibility antigens. The altered self theory proposes that the receptor acts as a single entity and recognizes the histocompatibility antigen, which can be considered as a marker for self, when it has been

modified by interacting with the foreign antigen.

Immunologists argued over these competing hypotheses for years, but could not resolve the issue without a clear view of the nature of the receptor. The cloned genes should be helpful in this regard, too. For example, they can be transferred independently to see how each alters the specificity of the recipient cells for foreign or histocompatibility antigen.

Meanwhile, Kappler and Marrack have evidence in favor of the altered self hypothesis. To prove that the first monoclonal antibody they identified was directed at the receptor molecule and not at some associated structure, they used it to screen some 400 additional T-T hybridoma clones. The antibody reacted with one with the same specificity for foreign antigen as the original clone against which the monoclonal antibody was directed. "The reaction with antibody predicted the specificity for antigen," Marrack says. The second clone also had the same cross-reactivity with histocompatibility antigens as the original, suggesting that a single receptor was recognizing both.

A complete T cell receptor may include more than the protein heterodimer identified by the monoclonal antibodies, however. "The interesting thing about the structure (the heterodimer) is that it could be demonstrated to be noncovalently linked in the membrane to what is now an old friend, T3," Reinherz says. T3 is the designation for a surface protein on all mature T cells that was identified by Reinherz and Stuart Schlossman of the Dana-Farber Cancer Institute in studies aimed at defining the surface molecules characteristic of the different types of normal and abnormal lymphoid cells. T3 appears late in differentiation, when the cells are becoming immunocompetent. It is apparently not directly involved in antigen recognition as its structure does not vary from clone to clone.

But antibodies against T3 block the antigen-specific activation of T cells. In addition, antibody against T3, which has been bound to Sepharose beads, activates T cells, just as the bound monoclonal antibodies to the heterodimers activated the appropriate cell targets. These results suggest, Reinherz says, that the T cell receptor for antigen is a complex of the cell-specific heterodimer with T3.

Additional proteins on T cell surfaces influence the reactivity of the cells with their targets. A molecule designated T4 (in the human, L3T4 in the mouse) is needed for recognition of class II histocompatibility antigens and one called T8 (or Lyt 2 in the mouse) is required for recognition of class I antigens. Antibodies to T4 and T8 do not block specific antigen-induced activation of T cells, indicating that they are not part of the receptor for antigen. However, they may increase the overall avidity with which the T cell binds to its target, perhaps by recognizing a framework region of the appropriate histocompatibility antigen or some other nonvariable component on the target cell surface.

In any event, T cell interactions with their partner or target cells are clearly complex. Their study will be greatly facilitated by the identification and isolation of the T cell receptor molecules and their genes, work which should supply the hitherto missing link of immune cell interactions. The immunoglobulin receptors of B cells have been well characterized by now. The histocompatibility antigens, which are among the target molecules recognized by T cells, are well on their way to being understood (*Science*, 27 May, p. 937). Soon the same may be said for the T cell receptor for antigen.

—JEAN L. MARX

Archeological Analysis Gets Some Teeth

A BASIC program has been developed that generates a mortality profile of ungulate species from tooth data in a fossil collection

Prehistoric sites frequently yield what even to the most experienced eye looks like an unpromising jumble of bones and stones. Without sound, objective methods of analysis there often remains considerable doubt over the paleontological and archeological significance of such sites. Focusing on teeth rather than bones, Richard Klein and Kathryn Cruz-Uribe of the University of Chicago have recently described* a technique that will help unjumble even some of the most meager collections of fossils.

The Chicago workers' specific interest with their technique is to be able to determine the age distribution (or mortality profile) of individuals of ungulate species represented in a fossil assemblage. Pat Shipman, a paleontologist at Johns Hopkins University, notes that obtaining reliable and detailed mortality profiles is extremely important in archeology and paleontology. "This technique," she comments, "gives a better shot at age profiles than anything else."

The basis of Klein and Cruz-Uribe's technique, which is a refinement of a model developed by British ecologist Clive Spinage, is the cumulative wear that teeth suffer through an animal's life. The use of teeth in establishing age profiles confers two distinct benefits.

First, as Shipman notes, the accuracy with which the degree of tooth wear can be measured compared with other age-related processes in the skeleton, such as fusion of epiphyses in bones, allows a much finer time resolution. "With tooth wear you can divide the lifespan into tenths as against only fifths or sixths with other methods."

The second benefit derives from the much higher rate of preservation of teeth in fossil deposits compared with other parts of the skeleton, which are softer and more likely to disintegrate. "Mandibles have an order of magnitude better preservation than other parts of the skeleton," says Shipman. "And single teeth have an order of magnitude better preservation than mandibles." The extension of age profile analysis to relatively impoverished fossil collection is therefore considerable using a technique that utilizes single teeth.

The ability to construct age profiles of fossil accumulations is crucial to an understanding of the nature of the accumulation, a perspective that was developed by Björn Kurtén and Leigh Van Valen in the 1950's and 1960's and more recently by Elizabeth Vrba at the Transvaal Museum in Pretoria, South Africa, and by Klein. As Klein and Cruz-Uribe explain in their recent *Paleobiology* paper, modern interpretations of age profiles refer to two theoretical models.

In one model the mode of death of the individuals in the assemblage is considered to be the result of some kind of

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