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Dissection of a Unique Tumor-Specific Transplantation Antigen into Multiple Unique Independent Epitopes using Syngeneic T-Cell Lines*

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In our past and present studies, we have analyzed the tumor-specific antigens and T-cell clonotypes that are involved in syngeneic tumor rejection. We expect that such information will be very useful for learning to manipulate tumor-specific immunity. As our tumor model, we have studied the murine ultraviolet-light (UV) induced fibrosarcoma 1591-RE and characterized its tumor-specific transplantation antigen using, as immunologic probes, syngeneic tumor-specific T-cell lines generated from animals that had rejected the tumor. This 1591 tumor, like many other UV-induced tumors, is very immunogenic and is routinely rejected by normal syngeneic mice [1]. We have previously shown that this resistance of the normal mice is dependent upon idiotypically restricted tumor-specific T cells [2, 3] which are specific for a unique 1591-speific tumor antigen. On rare occasions, normal mice develop progressively growing 1591 tumors, and these tumors (1591-PRO) are always heritable variants that have lost a unique regressor tumor-specific transplantation antigen [4]. (This loss has been determined by the resistance of the progressor variants to cytolytic T cells raised against the regressor tumor.)

In more recent studies, we have generated a syngeneic cloned cytolytic T-cell line that demonstrated the regressor-specific reactivity pattern, and we defined the epitope recognized by this T-cell clone as the "A" epitope [5]. We then used this cytolytic anti-A T-cell clone to select in vitro for tumor variants missing the A epitope in an attempt to dissect the tumor-specific transplantation antigen. The A^- variants were found in the 1591-RE population at a frequency of about 1 in 10^4 tumor cells, and these variants were not only resistant to the anti-A T-cell clone but also to cytoltic T cells from mixed lymphocyte-tumor cell bulk cultures (MLTC) responding to 1591-RE tumor cells. This suggested that the A epitope was present on a major tumor-specific transplantation antigen recognized by the host response, and that this antigen had been lost due to selection in vitro with the anti-A T-cell clone.

Similar to the in vivo derived variants, this A^- variant also grew progressively in normal mice at high doses and smaller doses of these variants could be rejected by normal mice. Interestingly, these mice which rejected A^- cells generated an immune response that lysed the A^- variants, and in agreement with our earlier studies [6], these anti-A^- bulk MLTC cells also lysed the original 1591-RE regressor tumor. The response was 1591-specific since no other UV-induced fibrosarcoma lines tested were lysed. We then derived T-cell lines from these MLTC cells, and, despite cloning, these T-cell lines retained a dual specificity pattern. This clearly indicated that the 1591-RE, the 1591-A^- variants, and the host selected 1591-PRO variant tumor cells all expressed one common tumor-specific epitope which was probably different from the A epitope. This epitope was, therefore, named the B epitope.

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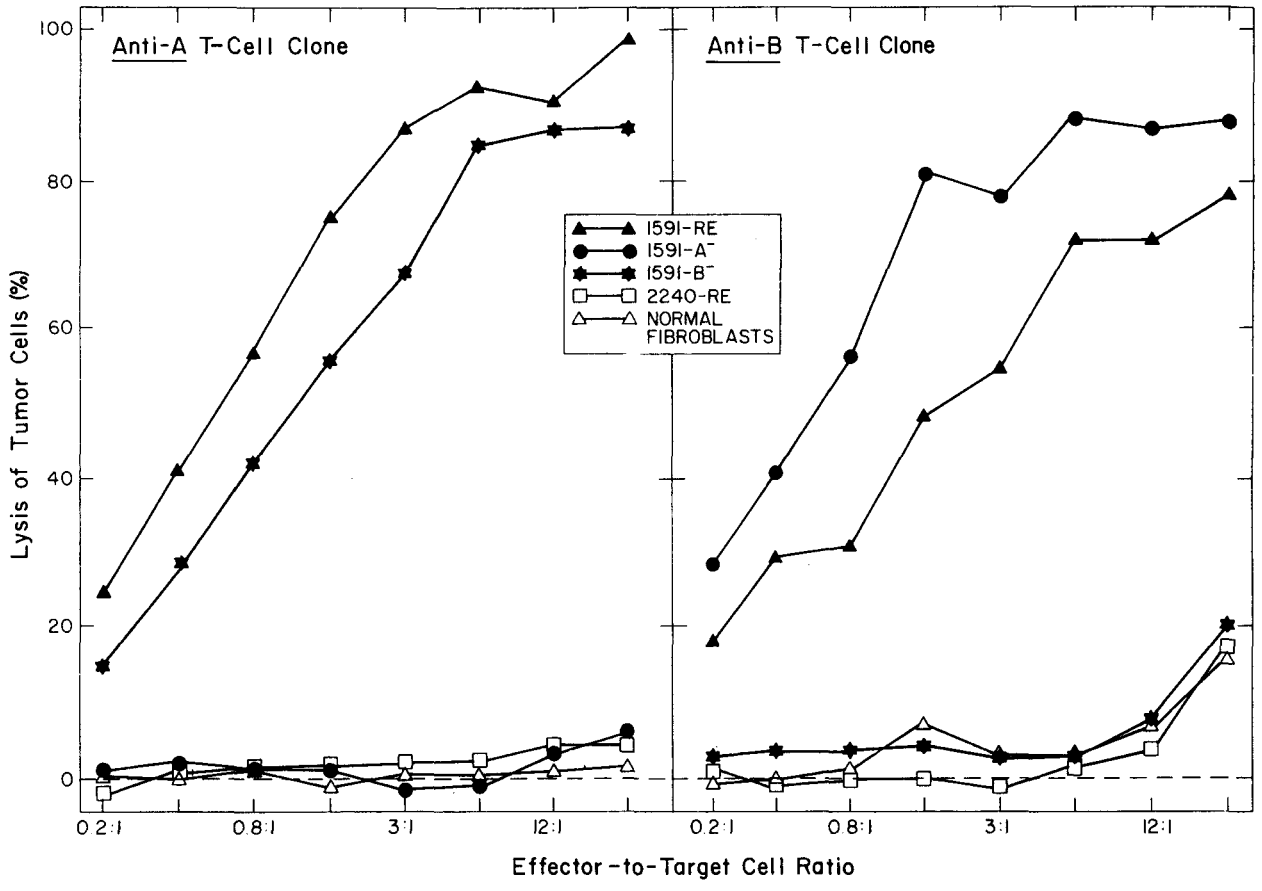

Fig. 1. Selective resistance of A^- and B^- 1591 tumor cell variants to either anti-A (*left*) or anti-B (*right*) T-cell clones as measured in a ^{51}Cr-release assay. For details see [5].

To further define the interrelationship of the two unique 1591-specific epitopes on the parental 1591 tumor, we determined next whether the loss of the A or the B epitope was independent or linked. Thus, we selected for 1591 tumor variants which were resistant to the anti-B T-cell line. We found that the B^- variants retained the A epitope while the A^- variants had retained the B epitope (Fig. 1). A similar "flip-flop" pattern was found upon analysis of the antigen dependence of the two T-cell lines (Fig. 2). The growth of both T-cell lines was stimulated by the 1591-RE cells, whereas 1591-A^- variant cells and 1591-B^- variant cells stimulated the T-cell line with the relevant specificity.

Six more variants derived by the same general protocol also showed independent loss of the A and B epitopes. This proves that the A and B epitopes are different and are not closely linked. The results are also inconsistent with the idea that the anti-B cell line is a high affinity clone for the A epitope. In other studies, which we will not elaborate on, we have found that there is still a third also unique 1591-specific epitope which we call the C epitope. Again, this epitope was defined by a syngeneic T-cell line and could be lost independently from the A and B epitopes. Obviously, if all these epitopes were expressed on one and the same antigenic molecule, we would have probably observed at least in some variants a simultaneous loss of more than one epitope after selection with a single cytolytic T-cell clone. Therefore, it appears more likely that these epitopes reside on different antigenic molecules.

At present, we can only speculate on the origins of multiple unique tumor-specific antigens suggested by the results of our studies. However, it is highly unlikely that they represent normal C3H histocompatibility antigens recognized by C3H mice due to "genetic drift" of the responder's histocompatibility antigens. If such genetic drift had occurred in the mice since the 1591 tumor had been derived, one would expect that the isolated T-cell probes would also react with the other UV control tumors which had been isolated at the same time,

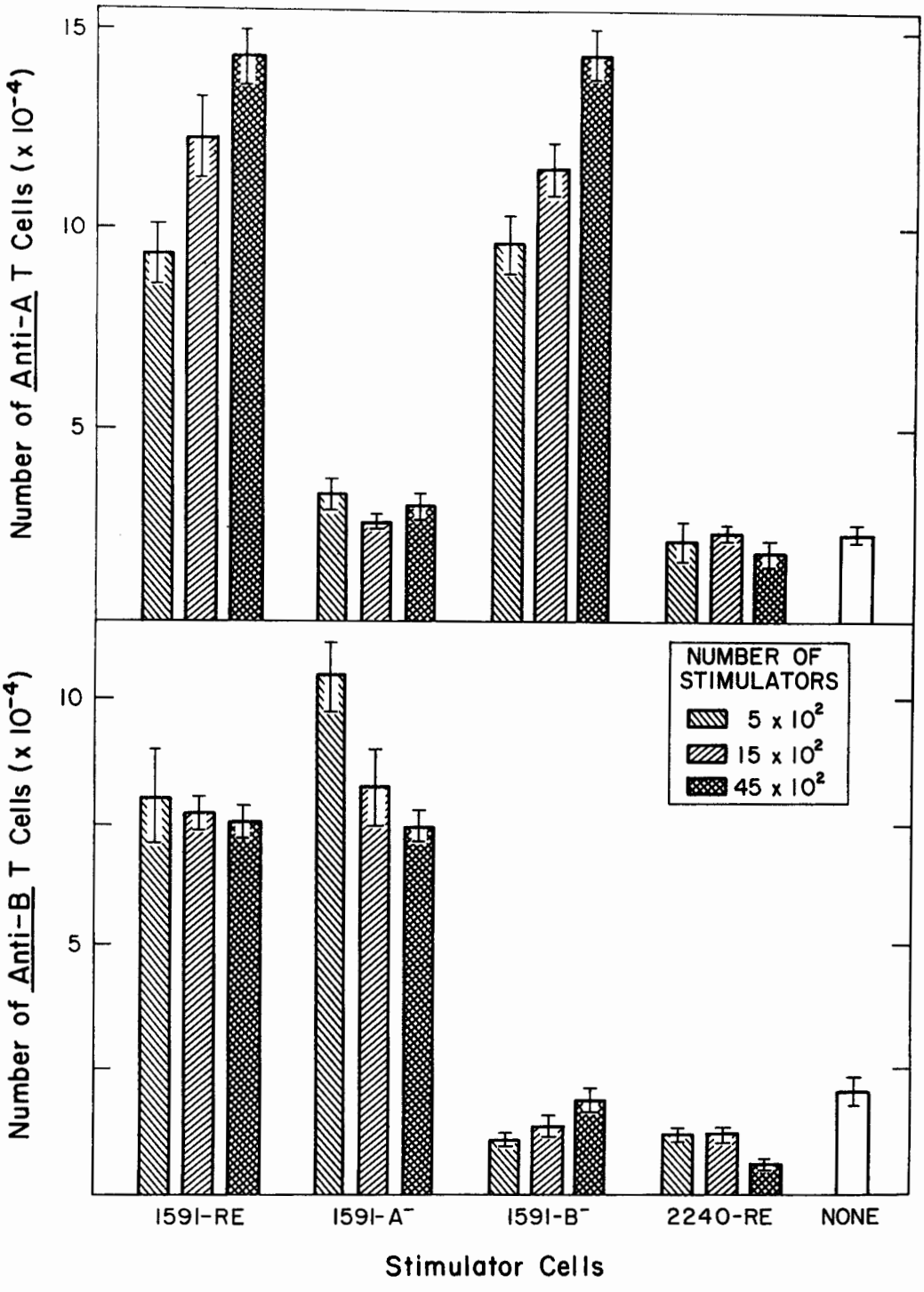

Fig. 2. Selective stimulation of anti-A (*upper*) and anti-B (*lower*) T-cell clones with either A^- or B^- 1591 tumor cell variants as measured by relative increase in T-cell numbers during a 7-day coculture with stimulator cells. For details see [5]

in the same experiment, and in the same stock of mice.

It is important to mention that despite this multiplicity of tumor-specific epitopes on the 1591 tumor cell, previous experiments have shown a clear hierarchy in the recognition of these epitopes [7]. The immune response of normal mice was always found to be directed to the A epitope, which was immunodominant over the immunorecessive B epitope; the B epitope was only recognized by the host once the A epitope had been lost from the 1591 cell. This hierarchy is in agreement with other earlier studies showing idiotypic restriction of the T-cell response to 1591-RE tumor cells. Obviously, the restriction of the immune response to certain immunodominant tu-

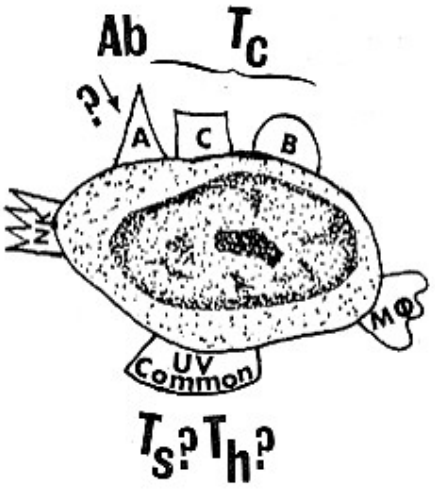

Fig. 3. Hypothetical scheme of the surface antigens on the parental 1591-RE tumor cell. While we consider it likely that the unique A, B, and C epitopes are on separate surface antigens, it is not clear whether the common UV antigen is part of an antigenic moiety common to all unique antigens, or a separate surface antigen. For details on the NK and macrophage-recognized target sites see [6, 7]

mor-specific epitopes on the malignant cell increases the chance of the tumor being able to escape immune destruction, because it only has to undergo one phenotypic change in order to escape immune destruction. On the other hand, it is clinically important that such variant tumors which have escaped the immune defense of the host probably retain other tumor-specific epitopes that can still act as targets for immunotherapy. Figure 3 shows a hypothetical schematic drawing of the antigenic surface makeup of the parental 1591- RE tumor cell. We have not discussed the data consistent with the existence of a different UV antigen which is common to all UV tumors. This antigen, which is included for completeness in Fig. 3, appears to be primarily recognized by regulatory antitumor immunity, such as that providing help or suppression [8, 9]. An analogous general functional separation of antigenic recognition appears to occur in allogeneic responses to so-called class I and class II histocompatibility antigens [10]. So far, we have not yet found evidence that the host can select against the expression of this common UV antigen. While, on the other hand, the multiple, unique tumor-specific epitopes discussed above appear to be the targets of cytolytic and possibly other types of immunoselective effector immunity and are sequentially selected against in an order which depends upon the hierarchy in the tumor-specific immune response.

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