Brief Definitive Report

ANALYSIS OF H-2 DETERMINANTS RECOGNIZED DURING THE INDUCTION OF H-Y-IMMUNE CYTOTOXIC T CELLS BY MONOCLONAL ANTIBODIES IN VITRO

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The cytotoxic T (Tc) cell response of female mice to the male-specific antigen (H-Y) is like many other antigen-specific Tc cells, restricted by the K and D regions of the H-2 complex (1). T helper (Th) cells restricted to the I region of the H-2 complex are a necessary auxilliary cell for the generation of Tc cells (2–4). An absolute requirement for Th cells mapping to the I-A subregion of the H-2^b haplotype has been proposed for the generation of H-Y-immune Tc cell responses (5). Experiments using irradiation (6, 7) and allophenic (8) chimeras were used specifically to show that CBA/H mice are nonresponders to H-Y because of a lack of help. However, we have shown that CBA/H mice can respond to H-Y when primed subcutaneously via the footpad. This immunization route may bypass the necessity for T cell help, which is otherwise required when immunization is performed via the conventional intravenous or intraperitoneal routes (9; M. Brenan and A. Müllbacher, manuscript submitted for publication).

Serological methods provide an alternative approach for analyzing H-2 determinants recognized during the induction of Tc cell responses. Monoclonal antibodies directed to H-2K^k and H-2D^k determinants have been shown to inhibit the effector function (10–12), as well as the induction of Tc cells (11). Recently, monoclonal antibodies directed to I^k have been shown to inhibit T-B collaborative responses (13). In this paper, we have used monoclonal antibodies specific for H-2-coded determinants of the H-2^k haplotype and show that K/D as well as I region-coded determinants are required for the generation of a CBA/H H-Y-immune Tc cell response.

Materials and Methods

Animals. CBA/H (K^k, I^k, D^k) and C3H.H-2° (K^d, I^d, D^k) mouse strains were obtained from the Clinical Research Centre animal breeding unit and used at 4–6 wk of age.

Immunizations. All mice were primed in the footpad with 10^7 nonirradiated syngeneic male spleen cells and used after a minimum of 4 wk after priming.

Antisera. The monoclonal antibodies 49.R1, 13/4, 27.R9, and 30.R3 (donated by Dr. G. Hämmerling, University of Cologne) were used. The properties of these antibodies have been described in detail (14, 15) and are summarized in Table I.

In Vitro Secondary Cultures (Memory Cultures). The generation of secondary H-Y-immune Tc cells has been described in detail previously (16). In brief, 4×10^5 nylon wool column separated spleen T cells (17) were cocultured with 10^5 irradiated (2,000 rad) syngeneic male spleen stimulators in a specified dilution of monoclonal antibody in 200 μl supplemented RPMI 1640

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TABLE I

Hybridoma	Immunization	Subregion	Designation of determinant	Approximate correlation with determinants defined by conventional sera
H 118-49.RI (49.RI)	BALB/c ∝ CBA	I-A	Ia.m1	Ia.2
13/4	A.TH ∝ A.TL	I-E	Ia.m8	Ia.7
H 100-27.R9 (27.R9)	BALB/c ∝ CBA	H-2K/H-25	H-2.m4	H-2.25
H 100-30.R3	BALB/c ∝ CBA	H-2K	H-2.m5	H-2.5

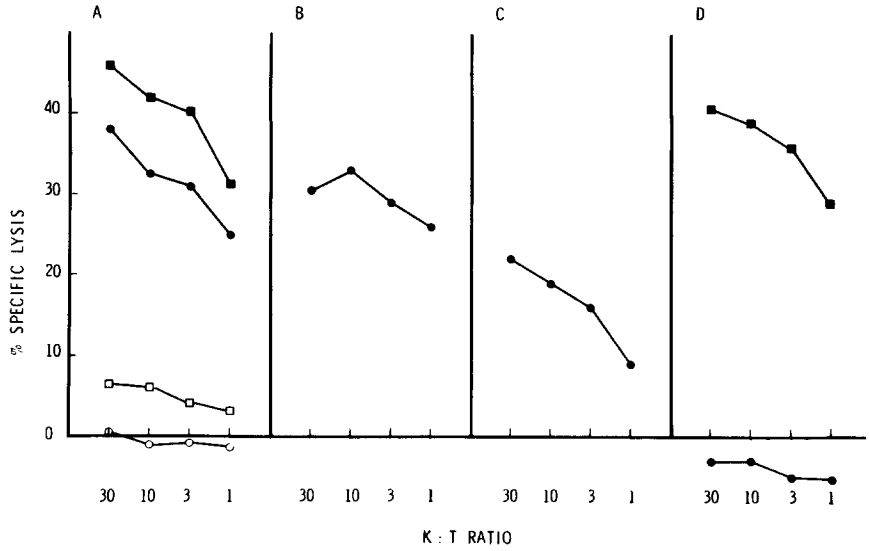

FIG. 1. Cytotoxicity of memory CBA/H (circles) and C3H.H-2° (squares) H-Y-immune Tc cells boosted in vitro with syngeneic male cells in the absence of antibodies (A), in presence of 49.RI (B), 13/4 (C), or 49.RI and 13/4 together (D), at a final concentration of 1:50 dilution in 200 μl. Effectors were assayed on male (closed symbols) and female (open symbols) syngeneic targets.

in 96-well round-bottomed microtiter Linbro plates (Linbro Chemical Co., Hamden, Conn.) for 5 d.

Target Cells. Target cells for H-Y-immune Tc cells were 24–48 h lipopolysaccharide (LPS) blasts prepared as described previously (16).

Cytotoxicity Assay. The method has been described in detail (16). The percent specific lysis was calculated by the formula:

$$\text{percent specific lysis} = \frac{\text{experimental release} - \text{medium release}}{\text{maximum release} - \text{medium release}} \times 100.$$

The spontaneous release ranged from 10 to 25%. Standard errors of the mean of the triplicates were <3% in the results shown in this paper.

Results

Effect of I Region-specific Monoclonal Antibodies on the Induction of CBA/H and C3H.H-2° H-Y Memory Tc Cell Responses. Nylon wool separated spleen T cells from individual mice primed in the footpad with syngeneic male spleen cells were used as responders in secondary in vitro cultures in the presence of specified monoclonal antibodies (Fig. 1). Control cultures (Fig. 1 A) contained no monoclonal antibody, and male target

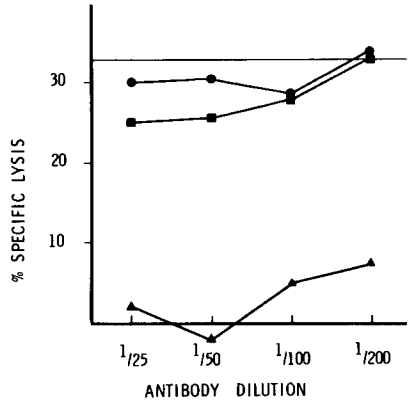

FIG. 2. Dose-response of monoclonal antibodies on induction of CBA/H H-Y-immune Tc cells. CBA/H H-Y memory T cells were boosted n vitro with syngeneic male cells in the absence (horizontal line) or presence of monoclonal antibody 49.RI (●), 13/4 (■), or 49.RI and 13/4 together (▲). Effectors were assayed on male CBA/H targets at a killer:target (K:T) ratio of 15:1.

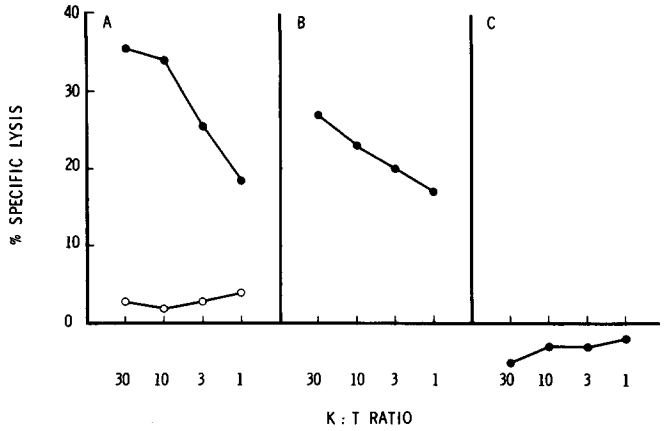

FIG. 3. Cytotoxicity of memory CBA/H H-Y-immune Tc cells boosted in vitro with syngeneic male cells in the absence of monoclonal antibodies (A) or in the presence of 30.R3 (B) or 27.R9 (C) monoclonal antibodies at a dilution of 1:50. Responders were assayed on male (●) and female (○) CBA/H targets.

cells were lysed efficiently. No significant inhibition occurred in the presence of antibody 49.RI (Fig. 1 B). 13/4 antibody gave partial inhibition at a 1:50 dilution (Fig. 1 C).

On the other hand, the presence of 49.RI and 13/4 together at the same concentration totally abolished the generation of CBA H-Y-immune Tc cells (Fig. 1 D).

Memory C3H.H-2° nylon wool-separated T cells were used to test the specificity of the inhibiton by 49.RI and 13/4 antibodies of CBA/H H-Y Tc cell responses. Fig. 1 A shows that spleen cells from C3H.H-2° mice in the absence of antibodies generate specific H-Y-immune Tc cells. The addition of 49.RI and 13/4 monoclonal antibodies together failed to inhibit the Tc cell responses (Fig. 1 D), which indicates that the inhibition of induction of CBA/H H-Y-immune Tc cells is specific for the I^k region.

To determine whether a true synergistic effect between 49.RI and 13/4 exists, serial twofold dilutions of antibodies (from 1:25 to 1:200) of either 49.RI or 13/4 alone or

together were added to cultures of CBA/H memory T cells. As shown in Fig. 2, good inhibition is obtained only in the presence of both antibodies. This extended over the entire range of dilutions used. In this particular experiment, no significant inhibition occurred with either of the two antibodies alone, even at their highest concentration.

Effect of K/D Region-specific Monoclonal Antibodies on the Induction of CBA/H H-Y-immune Tc Cells. CBA/H memory T cells were cultured in the absence (Fig. 3A) or in the presence of either 30.R3 (Fig. 3B) or 27.R9 (Fig. 3C) monoclonal antibody. Only 27.R9 inhibited the induction of CBA/H H-Y-immune Tc cells fully. Similar results were obtained using C3H.H-2° as responder cells (data not shown). These results confirm our earlier genetic mapping studies in which we have shown that CBA/H H-Y-immune Tc cells are restricted to the D end of H-2^k.

Discussion

The findings presented here clearly show that monoclonal antibodies directed to major histocompatibility complex products are well suited to investigate the fine specificity of T cell recognition during the induction of Tc cell responses in vitro.

We have shown previously by genetic mapping that homology between effector and target cell at the D end is necessary and sufficient for male specific target cell lysis by CBA/H H-Y-immune Tc cells (M. Brenan and A. Müllbacher, manuscript submitted for publication). This finding is confirmed by the ability to block induction of CBA/H as well as C3H.H-2° H-Y-immune Tc cells with antibody 27.R9, but not with antibody 30.R3. Only 27.R9 has specificity for D^k as well as K^k; 30.R3 shows specificity only for K^k.

However, the novel findings concern the attempt to block induction with antibodies directed to the I^k region, i.e., 49.R1 specific for I-A-coded determinants and 13/4 for I-E region-coded determinants. The results shown in Figs. 1 and 2 demonstrate that both antibodies (49.RI and 13/4) have to be present to achieve highly significant inhibition. Only marginal inhibition was observed with 13/4 alone in one experiment. A simple concentration effect could be ruled out from the dose-response data of Fig. 2, and it appears that there is a true synergistic effect of these two populations of antibodies similar to that observed in blocking studies at the effector level of influenza-immune Tc cells (12). The inability of these two antibodies together to block induction of C3H.H-2° H-Y-immune Tc cells shows their specificity for I^k and provides evidence that Tc cell responses to H-Y in CBA/H mice require a cell population during induction that recognizes I region-coded antigens, presumably Th cells.

C3H.H-2° and CBA/H possess the I-E-coded public specificity, Ia.7, recognized by monoclonal antibody 13/4. The finding that both antibodies have to be present at the same time to achieve significant blocking is consistent with the inability of 13/4 to block the induction of C3H.H-2° H-Y-immune Tc cells.

The simplest interpretation of the data concerning the inability of individual antibodies, either 13/4 or 49.R1, to block induction is that two subpopulations of H-Y-specific Th cells exist in CBA/H mice, one restricted by I-A^k and the other by I-E^k and I-A^k. Both populations of Th would individually be sufficient to augment a CBA/H H-Y-immune Tc cell response. The possibility that their combined effect is due to a binding on one Ia cell surface restriction antigen is less likely, as it is known that 49.R1 precipitates both Aα and Aβ polypeptides, both coded for in the I-A^k subregion, but not the Ae polypeptide, which is also coded for in the I-A^k subregion and which associates with Eα, coded for in I-E^k (18–20).

The findings by Hurme et al. (21, 22) that in H-2^b haplotypes, help for H-Y-immune Tc cell responses map to I-A^b exclusively, are consistent with our findings, as it is known that $E^b\alpha$ polypeptide chains are not expressed in this haplotype on cell surfaces (20).

We cannot exclude the possibility that only a single Th cell population recognizes an I-A^k and I-E^k cross-reactive determinant, although this seems unlikely to us, as T cells are generally regarded as having exquisite specificity for self major histocompatibility complex products (23).

In conclusion, we have shown the necessity for both K/D and I region-coded products for the induction of H-Y-immune Tc cells in CBA/H mice, thus strongly implying the requirement for T help. The role of two Th restriction elements is postulated.

Summary

Monoclonal antibodies directed to the D region of H-2^k when present during in vitro culture inhibit the generation of CBA/H and C3H.H-2° H-Y-immune cytotoxic T cells. Monoclonal antibodies directed to the I-A^k and I-E^k region specifically inhibited induction of CBA/H H-Y-immune cytotoxic T cells only when they were present simultaneously in culture. These findings show T helper cell requirement for CBA/H H-Y-immune cytotoxic T cell induction, and suggest that two I region-coded restriction antigens for T helper cells are involved.

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