Hybrid Antibody Mediated Veto of Cytotoxic T Lymphocyte Responses

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Summary

Strategies are being sought that allow the induction of specific tolerance to allogeneic transplants without affecting other immune functions. The so-called veto effect has been described as one such technology where $CD8^+$ cells suppress responses of class I MHC–restricted T-lymphocyte precursors to antigens expressed by those $CD8^+$ veto cells. Yet, veto inhibition will not be able to provide complete tolerance to allogeneic grafts since it only operates on cell populations that express CD8. Other types of cells prevalent in most organs express different tissue-specific antigens that are recognized by alloreactive T-cells. Therefore, complete tolerance to an allogeneic transplant can only be achieved if all cellular components within the graft acquire the immune-inhibitory function. Here, we studied whether the veto effect could be exploited for this purpose nevertheless. We produced a hybrid antibody (HAb) combining a mAb specific for a class I MHC molecule with a soluble CD8 molecule. We found that this HAb specifically and effectively transferred veto inhibition to different stimulator cell populations. Thus, we have developed a strategy that promises to selectively and completely tolerize graft-specific CTLs without affecting normal immune responses.

In a clinical situation, survival of allogeneic grafts is best achieved by the administration of immunosuppressive drugs, e.g., cyclosporine A, corticosteroids, etc. This regimen also impairs the patient's defenses against infectious challenges. Strategies are, therefore, being sought that successfully induce specific nonresponsiveness (tolerance) to the transplant. The veto effect has been described as one such mechanism in which $CD8^+$ cells suppress responses of class I MHC–restricted T-lymphocyte precursors to antigens expressed by those $CD8^+$ veto cells (Fig. 1 *A*) (1–3). The suppression is both antigen-specific (2, 3) and MHC-restricted (4), and results from the unidirectional recognition of the veto cell by the responding cytotoxic T-lymphocyte, but not vice versa (3, 4). It can be exerted by $CD8^+$ cells isolated from either bone marrow (BM)[1] or the spleen (2, 3, 5). This suppression functions in vitro and in vivo (6, 7), is very powerful in selectively removing T cells from the peripheral repertoire (8–12), yet does not affect negative selection in the thymus (13). Injection of veto cells has been shown to prolong graft survival in several animal models (10–12, 14). Class I MHC–restricted T cells with a wide spectrum of specificities to allo-antigens, minor and haptenated histocompatibility antigens have been inhibited by veto cells (6). Activated $CD8^+$ T cells represent the most active inhibitor population (6, 7). Veto activity is linked to the presence of the CD8 α-chains such that the veto function is lost if expression of CD8 is deleted, yet is reconstituted by reexpression of the CD8 α-chain (15). The function of $CD8^+$ veto cells is independent of their own recognition specificity (6, 7).

Activation of $CD8^+$ class I MHC–restricted T cells depends on coengagement of the TCR with the CD8 accessory molecule (16, 17). Disturbance of this interaction complex prevents T cell activation. Indeed, anti-CD8 mAb as well as antibodies (and peptides) blocking the CD8-binding site on class I MHC molecules inhibit the induction of T cells (18–20). Therefore, one could hypothesize that CD8 on stimulator cells, as in the veto effect, saturates CD8-binding sites on class I MHC molecules expressed by these cells. CTL precursors would be prevented from coengaging TCR and CD8 on the stimulators' class I MHC molecules. Yet, an alternative hypothesis has been offered. Experiments have been performed that suggest that T cells are deleted that have received a signal through their T cell receptor complex and at the same time through the α3 domain of their class I MHC molecules (21). Such a trigger is delivered by

[1] *Abbreviations used in this paper:* BM, bone marrow; HAb, hybrid antibody; SPDP, *N*-succinimidyl-3-(1-pyridyldithio)-propionate.

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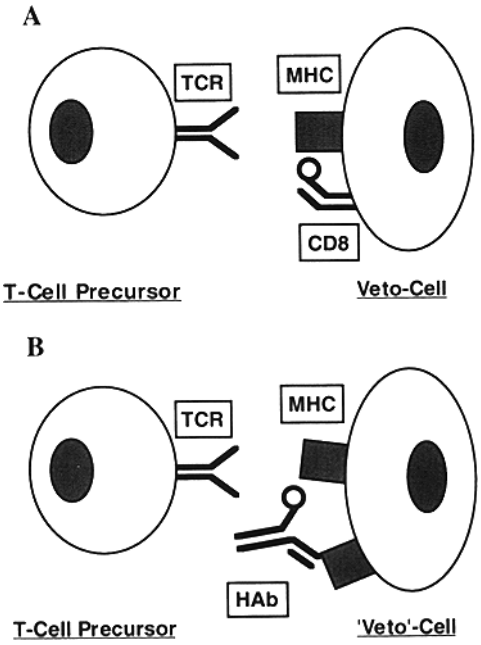

Figure 1. Schematic representation of HAb-mediated veto. (*A*) On conventional veto-cells the CD8 accessory molecule is endogenously expressed. (*B*) A HAb, here exemplified by 15-CD8, delivers CD8 to a cell surface via its binding to a class I MHC molecule.

CD8 on stimulator cells or by antibodies to this domain (21). Nevertheless, both theories predict that CD8 molecules delivered to any stimulator cell will veto reactive T cells.

Therefore, we produced a hybrid antibody (HAb) whose antibody moiety targets expression of the CD8 coreceptor onto cells (Fig. 1 *B*). Here, we demonstrate its ability to transform stimulator cells into inhibitory cells resulting in the specific suppression of class I MHC–restricted T cells. Thus, we have developed a strategy to specifically induce tolerance of class I MHC–restricted T cells.

Materials and Methods

Animals. Female mice (DBA/1, BALB/c, C57BL/6) 8 to 10 wk of age were purchased from the Jackson Laboratory (Bar Harbor, ME). T cell receptor transgenic (TCR_{trans}+) mice expressing the 2C-TCR_{trans} had been obtained from Dr. D. Loh (Washington University, St. Louis, MO), and were bred onto the permissive H-2^b background (22).

Hybrid Antibody Constructs. Dimers of the soluble human CD8 α-chain were harvested from CHO-DL cells transfected with the human CD8 α-chain truncated at position 146 (23). The cell line was maintained in serum-free, protein-free S2770-medium (Sigma Chem. Co., St. Louis, MO). Soluble CD8 was purified by a modification of the published method. Two (2) liters of tissue culture supernatant were filtered, adjusted to pH 8.5 and adsorbed to a Mono-Q-Sepharose column (Pharmacia, Uppsala, Sweden). The column flow-through was loaded onto a Wheatgerm-Lectin-Sepharose column (Pharmacia), and CD8 was eluted using 5% *N*-acetyl-glucosamine. The eluate was dialyzed against 20 mM $NaC_2H_3O_2$ (pH 5.4; Sigma), concentrated, and loaded onto Mono-S-Sepharose (Pharmacia) preequilibrated with 20 mM Hepes (pH 8.0; Sigma). Soluble CD8 was eluted as the first peak of a NaCl-gradient (20 mM to 500 mM; Sigma) in 20 mM Hepes (pH 8.0) and dialyzed against PBS. The purity of the CD8 α-chain preparation, as analyzed on SDS-PAGE, was higher than 99%. The mAb 15-5-5 is reactive with H-$2D^d$, yet not with H-2 molecules of the H-2^b and H-2^q haplotype (24). It was purified from tissue culture supernatant on a protein A–Sepharose column (Pharmacia) using standard protocols (25). Soluble CD8 and the mAb 15-5-5 were cross-linked as described previously with the help of the heterobifunctional cross-linker, *N*-succinimidyl-3-(1-pyridyldithio)-propionate (SPDP; Pharmacia) (25). Both 15-5-5 and CD8 were reacted with SPDP at pH 7.4. SPDP-modified proteins were separated from unreacted SPDP by Sephadex G25 column (Pharmacia) chromatography. Approximately one SPDP group was introduced per protein molecule. 2-pyridyl-disulphide groups of the mAb were reduced (at pH 5.0) to expose a reactive SH-group. Then, 15-5-5 was purified on a Sepharose G25 column and reacted with the modified CD8 at neutral pH. The hybrid antibody constructs, for short called hybrid antibodies (HAb) were analyzed on SDS-PAGE. Unless otherwise indicated the HAb and its components were used at final concentrations of 1 μg/ml.

Mixed Lymphocyte Culture and Cytotoxicity Assay. Spleen cells were harvested and single cell suspensions were prepared. Erythrocytes were lysed with Tris-buffered ammonium chloride (Sigma). Stimulator cells were irradiated (1,500 rad), then cultured (4×10^6 cells) with responder cells (2×10^6 cells) in one well of a flat-bottomed 24-well plate (Becton-Dickinson, Lincoln Park, NJ). Iscove's modified Dulbecco's medium (IMDM; Sigma) was used as growth medium supplemented with 5 mM Hepes, 2 mM glutamine, 1 mM hydroxypyruvate, 50 μM 2-mercaptoethanol, non-essential amino acids, 100 IU/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin (Sigma), and 10% fetal bovine serum (Intergen, Purchase, NY). The cultures were incubated for 4 d at 37°C and 7% CO_2 in a tissue-culture incubator (Forma Scientific, Marietta, Ohio). At termination of culture blast cells were counted. As indicated in the text, certain cultures received soluble CD8, 15-5-5 mAb or HAbs. To other cultures 2×10^5 irradiated (5,000 rad) P815 cells (BALB/c-derived mastocytoma cells) were added. These tumor cells had been precultured with soluble CD8 and 15-5-5 or HAbs at 1 μg/ml and had been washed before their introduction to MLCs. To test for cell-mediated lympholysis, effector cells were incubated with 1×10^4 [^{51}Cr]$NaCr_20_4$-labeled target cells, P815 or EL4 (C57BL/6-derived lymphoma cells) suspended in cell culture medium for 4 h in round-bottomed microtiter plates (total volume 0.2 ml; Becton-Dickinson). Varied numbers of effector cells were used resulting in different effector-target-ratios as indicated in the text. The plates were spun and the amount of ^{51}Cr-released into the supernatant was determined on an Automatic Gamma Counter (Micromedic, Hoorsham, PA). Wells containing target cells, but no effector cells were used to determine non-specific release, and wells containing target cells in the presence of 1% Triton X-100 (Sigma) to measure total release. Percent specific release was calculated as: [(cpm released in the presence of effector cells − cpm of non-specific release)/(cpm of total release − cpm of non-specific release)] × 100.

Flow Cytometry. T-cells were stained in single cell suspensions as described previously (26). They were incubated with biotinylated H57-597 (Pharmingen, San Diego, CA), which reacts with all αβ-TCRs, or with biotinylated 1B2, which specifically reacts with the $TCR_{idiotype}$, i.e., with the 2C-TCR_{trans}, followed by CyChrome-coupled streptavidin (Pharmingen). They were counter-stained on ice with phycoerythrin-coupled anti-CD4

and FITC-coupled anti-CD8 (Pharmingen). The extent of antibody binding to cells was analyzed on a FACScan® (Becton-Dickinson, San Jose, CA) flow cytometer. As indicated in the figures, data are given as $\log_{10}$ fluorescence.

Results and Discussion

Previously we had demonstrated that HAb both as heteroconjugates and as bi-specific monoclonal antibodies, were able to modify T cell responses (25, 27). We had shown that HAbs redirected lysis of cytotoxic T-lymphocytes (25, 27, 28) and induced T cell development in the thymus (29). Having established the versatility of the HAb approach, we decided to adapt this technology to transfer T cell veto. As the veto function resides within the CD8 accessory molecule, i.e., the CD8 α-chain, we coupled the CD8 α-chain to a mAb (15). Since the human CD8 functions within the mouse environment (30), we took advantage of a source of the human soluble CD8 α-chain that had been established by others to determine its crystal structure (23). As targeting entity, we selected a mAb with specificity to a mouse class I MHC molecule. This choice should enable the HAb to bind to virtually all cells. We chose the mAb 15-5-5 that reacts with class I MHC molecules of the H-2^d (H-$2D^d$), but not of the H-2^b or H-2^q haplotypes (24). To establish the feasibility of HAb-mediated veto, we decided to produce a bi-specific construct chemically cross-linking the two reagents (25). For this purpose the soluble CD8 α-chain homodimer was conjugated to the purified mAb resulting in a hybrid construct, here called HAb 15-CD8. In the first set of experiments, we tested whether 15-CD8 specifically guided the surface expression of CD8 α-chains. We coated P815 (H-2^d) and EL4 (H-2^b) cells with either 15-CD8 or with its non-conjugated components, the mAb 15-5-5 and the CD8 α-chain. The extent of CD8-surface expression was measured by staining with a fluoresceinated anti-CD8 mAb. As depicted in Fig. 2, neither 15-CD8 nor its components led to an appreciable surface expression of CD8 on EL4 (H-2^b) cells. Yet, 15-CD8 transferred large numbers of CD8 molecules onto P815 (H-2^d) cells. The non-conjugated components failed to do so. Thus, 15-CD8 had reacted according to the specificity of its antibody component, 15-5-5. It targeted expression of CD8 α-chains onto cells of the H-2^d, yet not the H-2^b haplotype.

Having established the binding specificity of 15-CD8, we examined whether T cell responses could also be inhibited. Mixed lymphocyte cultures (MLCs) were established with BALB/c (H-2^d) spleen cells as stimulators and C57BL/6 (H-2b) spleen cells as responders. In this strain combination, 15-CD8 selectively coats stimulator cells. The HAb and its non-linked components, CD8 and 15-5-5, were added at escalating concentrations into these MLCs. After 4 d of incubation, T-cells were harvested to determine their lytic activities towards their specific target, P815 (H-2^d). As depicted in Fig. 3, the HAb almost completely inhibited the induction of CTLs at concentrations as low as 125 ng/ml, whereas no significant effect was seen upon addition of the

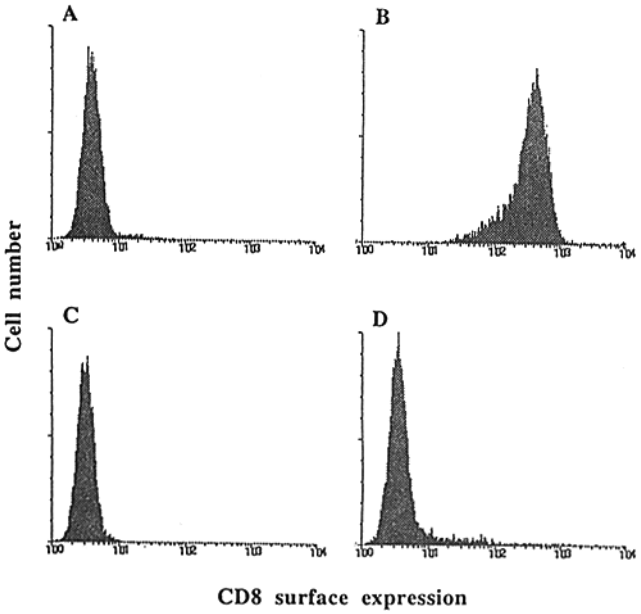

Figure 2. Specificity of 15-CD8 binding. P815 (H-2^d) (*A* and *B*) and EL4 (H-2^b) (*C* and *D*) cells were incubated either with the HAb 15-CD8 (*B* and *D*) or its non-linked components, the mAb 15-5-5 and the soluble CD8 α-chain (*A* and *C*). Surface expression of CD8 was determined with the help of a directly fluoresceinated anti-CD8 mAb.

non-linked components, CD8 and 15-5-5. Based on these results antibody concentrations were adjusted to 1 μg/ml in all other experiments. Next, we analyzed the functionality of the HAb in more detail. MLCs were supplemented with the HAb, soluble CD8, 15-5-5 or their combination. As shown in Fig. 4, addition of soluble CD8 and 15-5-5

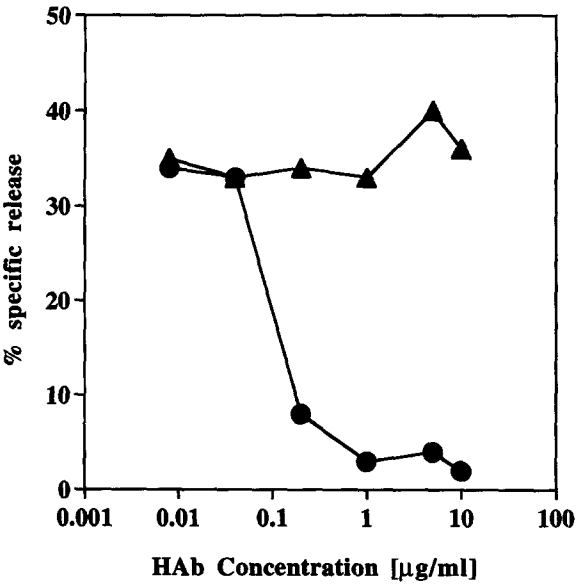

Figure 3. Concentration dependence of HAb-mediated inhibition of MLCs. Increasing concentrations of 15-CD8 (●) or its non-linked components (▲) were added to C56BL/6 (H-2^b) anti-BALB/c (H-2^d) MLCs. After 4 d cultures were harvested, and their lytic activities towards P815 (H-2^d) cells were tested.

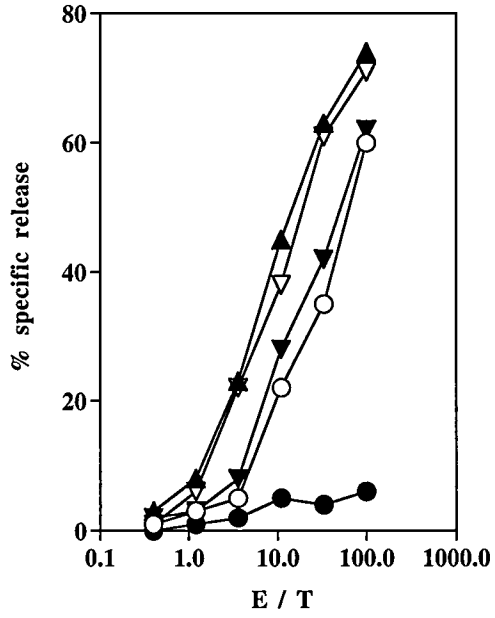

Figure 4. Ability of 15-CD8 to inhibit CTL induction. C57BL/6 (H-2^b) spleen cells were stimulated with irradiated BALB/c (H-2^d) spleen cells. These cultures were supplemented with the mAb 15-5-5 (▼), the soluble CD8 α-chain (▽), a mixture of the non-conjugated components (▲), or the HAb 15-CD8 (●). Controls were established in the absence of any of these reagents (○). After 4 d cultures were harvested, and their lytic activities towards P815 (H-2^d) cells were tested.

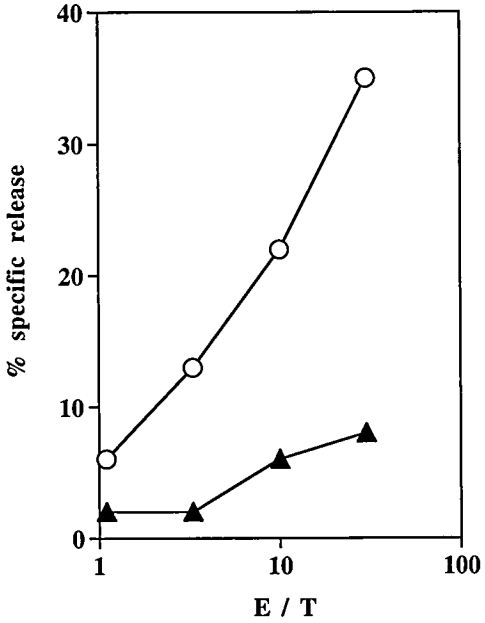

Figure 5. Ability of a hybrid construct to inhibit CTL activity induced by BM cells. A HAb was established using 15-5-5 F(ab')$_2$ fragments as targeting molecule. It was added to C57BL16 (H-2^b) spleen cells that were challenged with irradiated BALB/c (H-2^d) BM cells (▲). Control cultures were set up in the presence of the non-conjugated components (○).

alone or in combination did not impair the development of C57BL/6 T-cells into specific cytotoxic effector cells. Yet, 15-CD8 efficiently inhibited their stimulation to such extent that lytic activity towards the specific target, P815, could no longer be detected. From these results, we drew the following conclusions: (*a*) 15-CD8 suppressed CTL-activation in conventional MLCs. (*b*) The HAb components (the mAb, 15-5-5, and the CD8 α-chain) had to be linked to exert this inhibitory effect. The targeting MAb 15-5-5 specifically reacts with the H-2D^d class I MHC molecule expressed on H-2^d stimulator cells. Even if 15-5-5 were able to completely hide H-2D^d on BALB/c stimulator cells, it should not suppress responses directed towards other class I MHC molecules, i.e., H-2K^d and H-2L^d. Indeed, we did not observe that its addition significantly inhibited the induction of anti-H-2^d allo-reactive CTLs. It is also of interest that in this (Fig. 4) and several other experiments addition of the non-linked soluble CD8 alone or in addition to 15-5-5 resulted in a small increase in lytic activity. This finding might indicate that soluble CD8 competes with CD8$^+$ cells found within the splenic stimulators, thus, deleting their veto function.

As HAb-mediated inhibition might become useful in preventing rejection of BM grafts, we established a second MLC system in which BM cells acted as stimulator cells. CD8$^+$ cells normally found within normal BM populations were deleted from the BALB/c (H-2^d) BM population by antibody (anti-CD8) and complement treatment (31). Then, the remaining BM cells were used to challenge C57BL/6 (H-2^b) spleen cells. As depicted in Fig. 5, a HAb construct suppressed the induction of C57BL/6-derived CTLs. These findings again demonstrated the efficacy of HAb-mediated inhibition. Here, the inhibitory function had been transferred to BM rather than spleen cells. For this experiment, we had incorporated 15-5-5 F(ab')$_2$ fragments into the hybrid construct (27). Therefore, these studies also indicated that deletion of the Fc portion of the targeting mAb did not interfere with the inhibitory function. Thus, Fc receptor mediated binding of 15-CD8 was not crucial for the observed veto. Though these experiments provided some support for our notion that HAbs could transform non-lymphoid cell into veto cells, we addressed the validity of this postulate more directly in another experiment. For this purpose, irradiated P815 (H-2^d) mastocytoma cells were preincubated with the HAb or its non-linked components, and after washing they were added to C57BL/6 (H-2^b) anti-BALB/c (H-2^d) MLCs. When we measured the lytic activity of these cultures, we found that HAb-coated P815 cells had efficiently suppressed CTL responses (Fig. 6). However, P815 cells exposed to soluble CD8 and 15-5-5 failed to do so. As non-coated P815 did not decrease the observed CTL activity, we could exclude that the observed inhibition was due to competitive inhibition of the cytotoxicity assay by p815 itself. Therefore, these experiments demonstrated that HAbs could, indeed, convert non-lymphoid cells into veto cells.

We had already determined that 15-CD8 specifically bound to cells of the H-2^d haplotype, and that it was able to inhibit an allo-response. Yet, we had not determined

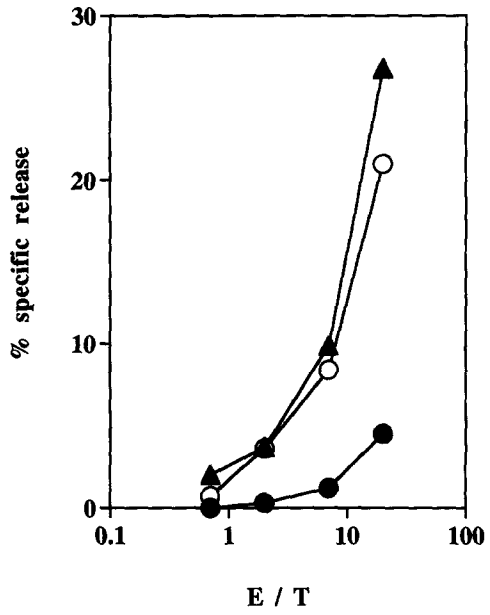

Figure 6. Transferring veto-function to non-lymphoid cells. Irradiated P815 (H-2^d) mastocytoma cells were preincubated with 15-CD8 (●), its non-linked components, CD8 and 15-5-5, (▲) or medium (○). After washing the treated P815 cells were added to C57BL/6 (H-2^b) anti-BALB/c (H-2^d) MLCs. After 4 d of culture their lytic actvities towards P815 cells were tested.

whether the inhibitory function exhibited similar specificity. As the targeting component of the HAb, mAb 15-5-5, does not bind to class I MHC molecules of the H-2^q haplotype (24), MLCs were established in which DBA/1 (H-2^q) cells were challenged by either BALB/c (H-2^d) or C57BL/6 (H-2^b) spleen cells. Whereas 15-CD8 bound to BALB/c H-2 stimulator cells, it could not link to MHC molecules on DBA/1 (H-2^q). When we added the HAb to these cultures, we observed that 15-CD8 selectively inhibited induction of DBA/1 (H-2^q) CTLs challenged with BALB/c (H-2d) spleen cells (Fig. 7 *A*). 15-CD8 did not suppress CTL activation when added to MLCs in which C57BL/6 (H-2^b) spleens cell had been used as stimulators (Fig. 7 *B*). Non-conjugated components of 15-CD8 did not affect lytic activities of either MLC. We had, therefore, demonstrated that 15-CD8 had to attach to cells to inhibit.

Yet, these experiments had not established that the HAb had to react with stimulator cells. To prove this point, two additional MLCs were set up, C57BL/6 anti-BALB/c (H-2^b anti-H-2^d) and BALB/c anti-C57BL/6 (H-2^d anti-H-2^b). In the first strain combination 15-CD8 coated stimulator cells, whereas in the second one it selectively linked to responder cells. As seen in Fig. 8, 15-CD8 selectively inhibited MLCs when binding to stimulator cells. However, in MLCs in which it was attached to responder cells vigorous CTL responses were induced. These experiments provided evidence that 15-CD8 had to bind to stimulator cells. Thus, the binding specificity of the hybrid construct predicted the direction of its inhibitory function. The unidirectional nature of these results also supported our earlier contention that Fc receptor-mediated binding, if it occurred, was not crucial for the inhibitory function exhibited by the HAb. Fc receptors are expressed by cells found within both BALB/c and C57BL/6 spleens, yet the HAb selectively suppressed CTL precursors specific for BALB/c cells.

The experimental system employed by us differed in one additional aspect from that of previous studies: other studies had used non-irradiated cells as inhibitory agent because it had been noted that the veto-function of $CD8^+$ cells was radiosensitive (2, 3). In our system, by contrast, 15-CD8 was perfectly capable of inhibiting CTL responses when binding to irradiated stimulator cells (Fig. 9). Indeed, the extent of suppression was comparable to that observed using non-irradiated cells. If we assume that HAb-mediated inhibition and conventional veto function by the same mechanism, this difference in results can be best explained as follows. Most investigators studying conventional veto

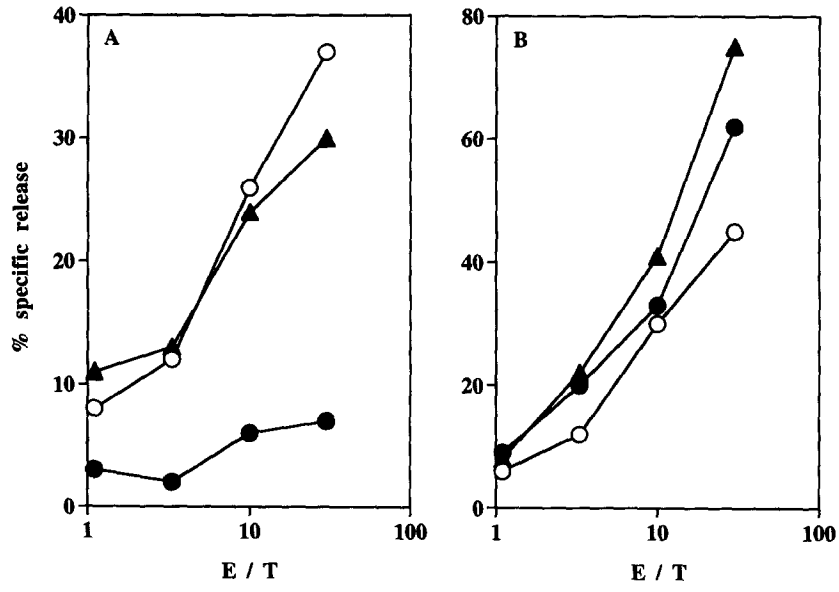

Figure 7. Dependence of HAb activity on its ability to bind to cell surfaces. Two different MLCs were set up: DBA/1 (H-2^q) anti-BALB/c (H-2^d) (*A*) and DBA/1 anti-C57BL/6 (H-2^b) (*B*). They were supplemented with the HAb (●), its non-conjugated components (▲) or nothing (○) and tested for their lytic ability on P815 (H-2^d) (*A*) or EL4 (H-2^b) (*B*) cells.

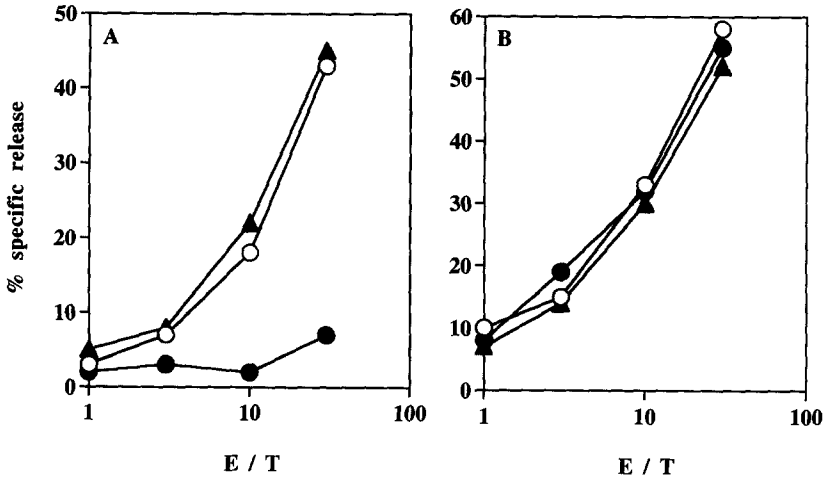

Figure 8. Specificity of HAb-mediated veto. MLCs were established in two directions: C56/BL6 (H-2^b) anti-BALB/c (H-2^d) (*A*) and BALB/c anti-C57BL/6 (*B*). They were supplemented with HAb (●), its non-conjugated components (▲) or nothing (○) and tested for their lytic ability on P815 (H-2^d) (*A*) or EL4 (H-2^b) (*B*).

exploited the inhibitory function of $CD8^+$ CTLs which are known to be radio-sensitive. Irradiated T-lymphocytes used as veto cells in vitro are deleted early. They are, therefore, not able to inhibit allo-reactive T cells that had remained dormant and are stimulated at a later time. HAbs have the ability to transfer CD8 to all stimulator cells, including those that are rather radio-resistant, e.g., macrophages and fibroblasts. Thus, CD8-bearing cells are present throughout the entire culture period, and have the ability to veto, both T cells stimulated early as well as those activated late. Our conclusion that HAb-mediated veto can be mediated by, for instance, macrophages and dendritic cells, also implies that CTL lytic functions are not crucial for T cell mediated veto.

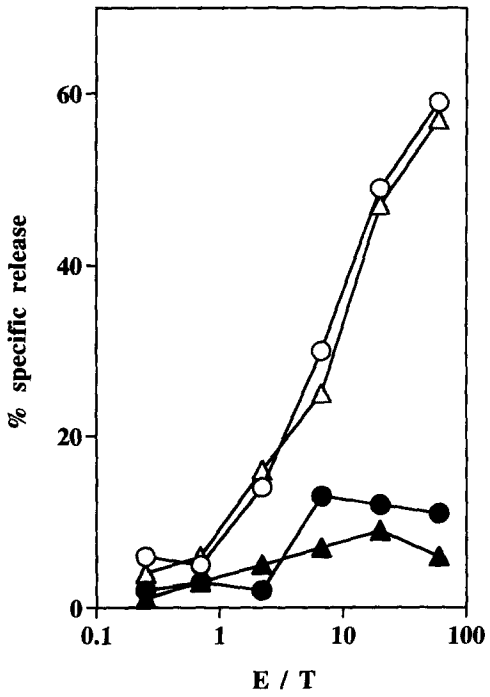

Figure 9. Radio-resistence of HAb-mediated veto. C57BL/6 (H-2^b) cells were incubated with non-irradiated (○) or irradiated (△) BALB/c (H-2^d) spleen cells. Cultures were supplemented with 15-CD8 (●, ▲) or with the non-conjugated components (○, △) and tested for lysis of P815 (H-2^d) cells.

To study the fate of T cells in HAb-mediated MLCs, we took advantage of a TCR transgenic mouse model in which the vast majority of T cells expressed the 2C-TCR_{trans} which recognizes the allo-class I MHC molecule H-$2L^d$ (22). In these mice TCR_{trans}+ T cells can be identified by the anti-clonotypic mAb.1B2. Responder TCR_{trans}+ T cells were stimulated with irradiated BALB/c spleen cells in the presence of 15-CD8 and its non-conjugated components. Numbers of TCR_{trans}+$CD8^+$ T cells were enumerated at the start as well as at the termination of cultures (Table 1). We failed to detect an increase in the cellularity of cultures supplied with the hybrid constructs. Thus, 15-CD8-mediated similar to conventional veto had prevented the expansion of reactive T cells (13, 21).

Based on these observations, we believe that conventional veto and HAb-mediated inhibition function through the same mechanism. Yet, HAb-induced unresponsiveness might bear some advantages over conventional veto. Although the conventional veto effect is extremely efficient, it only operates on $CD8^+$ cells, i.e., on defined populations

Table 1. *Inhibition of TCR_{trans}+$CD8^+$ T cells by HAb engagement*

	Number of TCR_{trans}+$CD8^+$ T cells	
Compound added	Experiment 1	Experiment 2
15-5-5 and CD8	6.1×10^4	6.0×10^4
15-CD8HAb	1.2×10^4	1.8×10^4

2×10^4 TCR_{trans}+$CD8^+$ T cells were added to 1×10^6 irradiated BALB/c (H-2^d) spleen cells in the presence of the compounds indicated. After 4 d cultures were harvested, cells were counted and stained for expression of CD4, CD8, and TCR_{trans} in three-color immunofluorescence. From these informations the numbers of TCR_{trans}+CD8 T cells were calculated.

of inhibitor cells (2). It has to be taken into consideration that different types of cells express unique tissue-specific antigens (32) and that some allo-reactive T cells recognize these differences (33–35). Thus, allo-responses discriminate between different tissues. Therefore, allo-reactive T cells exhibiting tissue-specific recognition cannot be silenced by the infusion of, for instance, $CD8^+$ T cells as they fail to present antigens typical for other cell types. Complete tolerance to an organ transplant is, therefore, not induced by cells of a single type, but is best achieved if all cells within the graft are transformed into inhibitory cells. As we have demonstrated that non-lymphoid cells can acquire the veto function, HAbs will provide the necessary tool to achieve this goal. Thus, we have developed a technology that promises to provide specific and complete transplantation tolerance without affecting other immune functions.

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