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Selective and unidirectional membrane redistribution of an H-2 antigen with an antibody-clustered viral antigen: Relationship to mechanisms of cytotoxic T-cell interactions

(H-2 restrictions/*syn*-capping/cellular immunology)

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ABSTRACT **We have studied the co-redistributions of vesicular stomatitis virus (VSV) antigen and of individual H-2 antigens on the surfaces of mouse cells, and in parallel we have also used these VSV-infected cells as targets in cytotoxic T-cell killing experiments. Antibody-induced patching and capping of the VSV antigen caused an extensive co-patching and co-capping of the H-2K^b antigen but not of the H-2D^b antigen. In reciprocal experiments, the antibody-induced patching of the H-2K^b or H-2D^b antigen did *not* result in a co-patching of the VSV antigen. Radioimmunoassays showed that the relative numbers of H-2K^b, H-2D^b, and VSV antigens on the surfaces of the cells exhibiting such nonreciprocal co-redistributions were closely similar. Furthermore, the H-2 restricted cytotoxic T-cell lysis of these target cells showed a marked preference for H-2K^b compared to H-2D^b compatibility. We propose that the VSV and H-2 antigens are molecularly independent entities in the unperturbed target cell membrane but that the antibody-induced clustering of the VSV antigen causes a selective and unidirectional co-redistribution (which we designate as *syn*-capping) of H-2K^b with the VSV antigen clusters. It is suggested that such a T-cell-induced *syn*-capping process involving an antigen and an H-2 molecule on the target cell may play a critical role in the mechanism of cytotoxic T-cell killing.**

Specific cell–cell interactions in immune systems involve a complex recognition system in which an effector cell recognizes two molecular features on the surface of a target cell: a foreign antigen, and a gene product of the major histocompatibility complex (MHC) (1, 2). In the case of cytotoxic T-cell killing of target cells in the mouse, the H-2K and H-2D histocompatibility antigens are the primary MHC products that are involved (3–6). Thus, a cytotoxic T cell will kill only those target cells that have on their surfaces the specific H-2 molecule and a foreign antigen A that were also present on the cell used to stimulate the immune response. The requirement for the appropriate H-2 molecule is known as H-2 restriction. The molecular mechanisms that operate in such complex recognition are not known, but two possible classes of mechanisms have been proposed. In one, it is postulated that there is only a single recognition molecule (receptor) on the surface of the cytotoxic T cell, which recognizes a neoantigen formed by the molecular interaction of the antigen A and an H-2 molecule on the surface of the target cell. This class of mechanisms is termed "single recognition" (or "altered self"). The other class, called "dual recognition," proposes that there are two different receptor sites on the cytotoxic T cell, one recognizing the H-2 molecule and the other the antigen A.

The structural relationships between H-2 molecules and various other antigens in a target cell membrane are therefore of critical importance in understanding cytotoxic T-cell killing. This problem has been studied in several cases (7–11) by carrying out antibody-induced redistributions (capping) of either an H-2 antigen or a foreign antigen A on a cell surface and determining whether the second component has redistributed (co-capped) with the first. In the present paper, we have examined in more detail the mechanisms of such co-capping of H-2K or H-2D antigens and a viral antigen (the VSV-G glycoprotein) on the surfaces of mouse cells infected with vesicular stomatitis virus (VSV); in parallel, the H-2K and H-2D restrictions in the cytotoxic killing of these VSV-infected cells were determined. The implications of our results and their relevance to the mechanism of cytotoxic T cell interactions are discussed.

MATERIALS AND METHODS

Cells and Tissue Culture. Fibroblast line MC57G (haplotype *H-2^b*), lymphoma line EL4 (*H-2^b*), and mastocytoma P815 (*H-2^d*) were maintained in culture in minimal essential medium containing fetal calf serum (10% for the first line and 5% for the other two lines). The cells were infected with VSV Indiana or VSV New Jersey strains (kindly provided by J. J. Holland, Department of Biology, University of California at San Diego, La Jolla, CA) at a multiplicity of infection of 100 for MC57G cells and 50 for EL4 and P815 cells.

Immunochemical Reagents. Rabbit antisera to VSV Indiana or to its purified VSV G-glycoprotein, were obtained from J. J. Holland. The specificity of the antisera was demonstrated by their inability to stain either uninfected cells or cells infected with VSV New Jersey (see Fig. 1 *q* and *s*), the G-glycoprotein of which does not serologically crossreact with that of VSV Indiana. In the experiments reported in this paper, similar results were obtained with both antisera. Alloantisera specific to individual H-2 antigens were produced by immunization with cells from spleen, lymph nodes, and thymus as reported (12). The sera used, numbered *K-333* (specific for H-2K^b antigen 33 and IA antigen Ia20); *K-302* (specific for H-2D^b antigen 2); and *K-304* (specific for H-2D^d antigen 4), were as described (12). The serum *K-548* (specific for H-2K^d antigen 31 and IA antigen Ia 11) was produced by immunizing (B10.A × A/J)F_1 mice with B10.D2 cells. The specificities of these sera for their respective H-2 antigens were demonstrated by their inability to stain allogeneic cells (as in Fig. 1*v*). Goat antibodies to pure IgG of mouse and rabbit were isolated by affinity chromatography on Ultrogel-bound IgG (13) of the homologous species and then absorbed on the heterologous IgG-immunoadsorbent to eliminate any crossreactivity. Rhodamine and fluorescein conjugates of the goat antibodies were prepared by published procedures (14).

Immunofluorescence. Viable cells were indirectly immunostained under capping conditions for VSV or for individual

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Abbreviations: MHC, major histocompatibility complex; VSV, vesicular stomatitis virus.

H-2 antigens as described (15), and the cells were then fixed in 3% paraformaldehyde in phosphate-buffered saline containing 1 mM Mg^{2+} and 0.1 mM Ca^{2+}. They were then immunostained for the second antigen by using the second fluorophore and examined with a Zeiss Photoscope III (14, 15).

Quantitative Radioimmunolabeling of VSV and H-2 Antigens. Cells (5×10^5) in monolayers (MC57G) or in suspension (EL4) were incubated at 0°C for 15 min with anti-VSV or anti-H-2 antisera at different dilutions in 0.2 ml of phosphate-buffered saline containing 10% calf serum and 0.01% NaN_3. The cells were then washed and incubated for 15 min with saturating amounts of ^{125}I-labeled goat antibodies to mouse IgG or goat antibodies to rabbit IgG (20 μg, 10^6 cpm in 0.2 ml). The cells were subsequently washed, lysed with 0.1% sodium dodecyl sulfate and assayed for radioactivity. The numbers listed in Table 1 are the saturation values obtained in the limit of high concentrations of the anti-VSV and anti-H-2 antibodies. Nonspecific labeling was measured by replacing the antisera with nonimmune sera.

Cytotoxicity Assay. Mice were infected intravenously with VSV Indiana at 5×10^7 to 10^8 plaque-forming units per animal. They were sacrificed 6 days later, their spleens were removed, and the spleen cells were tested for cytotoxicity against ^{51}Cr-labeled, VSV-infected target cells as described (16, 17).

Inhibition of Cytotoxicity by Alloantisera. The ^{51}Cr-labeled, 2-hr VSV-infected MC57G ($H\text{-}2^b$) target cells (5×10^4 cells in 50 μl) were first mixed with 25 μl of antiserum dilution. After 30 min at 24°C, C57BL/6 ($H\text{-}2^b$) effector cells (2×10^6 cells in 125 μl) were added and the cytotoxicity assay was carried out as above.

RESULTS

Effect of VSV Infection on Cell Surface Expression of VSV and H-2 Antigens. In order to interpret the capping studies described below, it was necessary to know the relative numbers of VSV-G and H-2 antigens on several of the infected cells that were studied. VSV infection shuts off host protein synthesis, and it is known (18) that, with time after infection, the ratio of surface-expressed VSV to H-2 antigens increases. This was also shown in the present experiments, as determined by indirect radioimmunolabeling of intact cells (Table 1). One hour after infection of EL4 cells, the relative numbers of VSV-G, H-$2K^b$, and H-$2D^b$ molecules were comparable (a point to be stressed below). After 3 hr, however, marked parallel decreases in H-$2K^b$ and H-$2D^b$ were noted, with a simultaneous 8- to 10-fold increase in the expression of VSV-G antigen. Similar results were obtained with the VSV-infected MC57 cells.

Effects of Redistribution of VSV Cell Surface Antigen on Distributions of H-2 Antigen. The antibody-induced surface redistribution of VSV-G antigen on MC57G cells (a fibroblast line) was less extensive and slower than on EL4 and P815 cells. Under conditions such that optimal redistribution of VSV-G was induced, the observed distributions of H-2 antigens on VSV-infected cells were as shown in Fig. 1. The H-$2K^b$ antigen was largely associated with VSV-G patches on MC57G cells 3 hr after infection (Fig. 1 *a* and *b*) and VSV-G caps on EL4 cells 1 hr after infection (Fig. 1 *e* and *f*), and markedly depleted from the remaining surfaces of these cells. On the other hand, the H-$2D^b$ antigen largely remained uniformly distributed and showed only slight enrichment in areas where the VSV-G antigen was patched (Fig. 1 *c* and *d*) or capped (Fig. 1 *g* and *h*).

Table 1. Radioimmunolabeling of VSV and H-2 antigens on VSV-infected cells and uninfected controls

Cells	Net cpm bound/10^6 cells* anti-VSV	anti-H-$2K^b$	anti-H-$2D^b$
EL4, not infected	640	16,500	12,100
EL4 VSV-infected, 1 hr	11,500	12,500	10,800
EL4 VSV-infected, 3 hr	64,500	3,540	2,870
MC57G, not infected	1,050	10,550	12,480
MC57G VSV-infected, 1 hr	21,500	8,590	10,260
MC57G VSV-infected, 3 hr	76,100	4,410	4,640

* Number of cells was verified by direct counting prior to the determination of cell-associated radioactivity. The values represent the mean of duplicate samples. Nonspecific binding (obtained with nonimmune sera) was low (375 for rabbit serum and 430 for mouse serum) and was subtracted from the experimental values. The numbers represent saturation values obtained in the limit of high concentrations of anti-VSV or anti-H-2 antisera.

With 3-hr VSV-infected cells of the $H\text{-}2^d$ haplotype (P815), the capping of VSV-G antigen did not result in a significant co-redistribution of either the H-$2K^d$ (Fig. 1 *m* and *n*) or the H-$2D^d$ (Fig. 1 *o* and *p*) antigens.

Effect of Redistribution of H-2 Antigens on the Distribution of VSV-G Antigen. In these reciprocal experiments, only the EL4 cells 1 hr after VSV infection were studied because they expressed comparable amounts of the H-$2K^b$, H-$2D^b$, and VSV-G surface antigens; the great excess of VSV-G over H-2 after 3-hr infection rendered reciprocal co-capping experiments with these cells uninterpretable. The patching and capping of H-$2K^b$ (Fig 1*j*) or of H-$2D^b$ (Fig. 1*l*) did not result in any significant co-redistribution of VSV-G antigen (Fig. 1 *i* and *k*, respectively) on 1-hr-infected EL4 cells. Similar experiments with 1-hr-infected MC57G cells were difficult to analyze because the same indirect antibody treatment of H-$2K^b$ or H-$2D^b$ resulted in only a finely punctate clustering of these antigens (not shown) instead of the extensive patching observed with EL4 cells.

Thus, the co-capping of H-2 and VSV-G antigens appears to be both *selective* (H-$2K^b$ but not H-$2D^b$, H-$2K^d$, or H-$2D^d$ co-caps extensively with VSV-G antigen) and *unidirectional* (whereas H-$2K^b$ associates with caps of VSV-G, VSV-G does not associate with caps of H-$2K^b$).

Cytotoxic T-Cell Lysis of VSV-Infected Cells. Studies were conducted to determine if the H-2-restricted lysis of VSV-infected target cells showed a preference for *H-2K* or *H-2D* compatibility. Spleen cells obtained from strains of mice with different *H-2* haplotypes, infected 6 days earlier with VSV, were tested on VSV-infected target cells in a ^{51}Cr release assay (16, 17). The results (Fig. 2*A*) show that lysis of VSV-infected MC57G (K^bD^b) cells depended primarily on compatibility at $H\text{-}2K^b$. Effector spleen cells from B10.A(5R) mice (K^bD^d) were as efficient as those from C57BL/6 (K^bD^b) or BALB/B (K^bD^b) in lysing the infected MC57G targets, but effector cells from B10.HTG (K^dD^b) mice were about $^1/_{10}$th as efficient. Spleen cells from VSV-infected allogeneic mice (BALB/c, K^dD^d) produced virtually no lysis of the infected MC57G target cells. Similar results (not shown) were obtained with VSV-infected EL4 (K^bD^b) cells as targets.

Examination of the same effectors on VSV-infected P815 cells (K^dD^d) demonstrated that there was no marked difference in cytotoxic efficiency between spleen cells obtained from mice compatible only for $H\text{-}2K^d$ or $H\text{-}2D^d$. Thus, as illustrated in Fig. 2*B*, spleen cells from both B10.A(5R) mice (K^bD^d) and B10.HTG mice (K^dD^b) lysed VSV-infected P815 cells as efficiently as did BALB/c (K^dD^d) cytotoxic T cells. Again, background levels of lysis were obtained by allogeneic C57BL/6 or BALB/B (K^bD^b) cytotoxic T cells on P815 targets (Fig. 2*B*). The maximal efficiencies of cytotoxic lysis that were achieved were greater when directed against the $H\text{-}2^b$ haplotype than against the $H\text{-}2^d$.

Antibody Inhibition of Cytotoxic T-Cell-Mediated Lysis of Target Cells. A further test of a preference for *H-2K* or

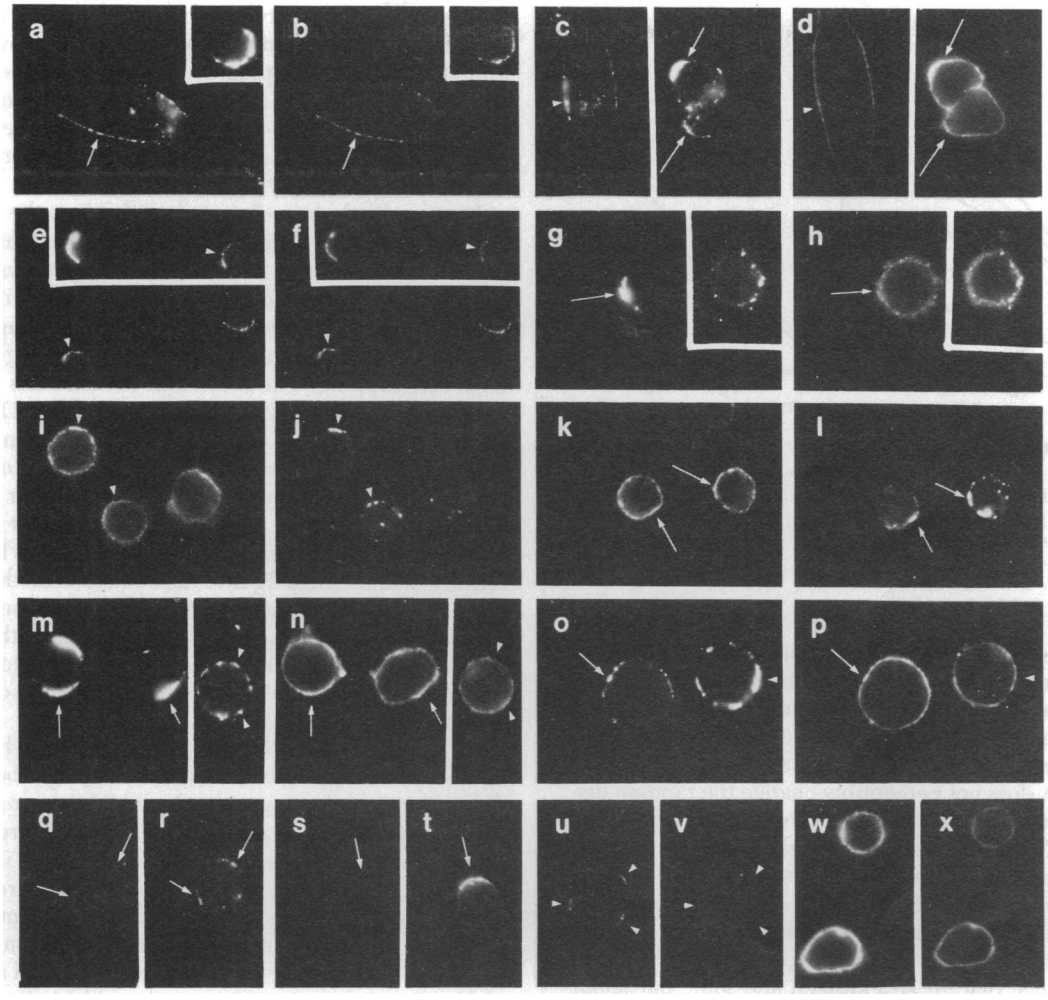

FIG. 1. Co-redistribution experiments with VSV and H-2 cell surface antigens. (*a–d*) MC57G cells (K^bD^b) 3 hr after infection with VSV Indiana: After VSV G-protein was first redistributed into patches by fluorescein-labeled indirect antibody treatment (*a* and *c*), the cells were fixed and then stained by rhodamine-labeled indirect antibody for the H-2K^b antigen (*b*) or the H-2D^b (*d*) antigen. In *b* the H-2K^b antigen has nearly completely co-redistributed with the VSV-G protein in *a*, whereas the H-2D^b antigen in *d* has not significantly co-redistributed with the VSV-G protein in *c*.

(*e–h*) EL4 cells (K^bD^b) 1 hr after infection with VSV Indiana. After the VSV-G protein was capped by fluorescein-labeled indirect antibody treatment (*e* and *g*), the cells were fixed and then stained by rhodamine-labeled indirect antibody for the H-2K^b antigen (*f*) or the H-2D^b antigen (*h*). The H-2K^b antigen in *f* has nearly completely co-capped with the VSV-G protein in *e*, whereas the H-2D^b antigen in *h* has not significantly co-capped with the VSV-G protein in *g*.

(*i–l*) The reciprocal experiments to those in the second row, with EL4 cells 1 hr after infection. The H-2K^b antigen (*j*) or the H-2D^b antigen (*l*) was first capped by rhodamine-labeled indirect antibody treatment, then the cells were fixed and stained by fluorescein-labeled indirect antibody for VSV-G protein (*i* and *k*, respectively). The VSV-G protein in *i* and *k* did not significantly co-redistribute with either the caps of H-2K^b (*j*) or the caps of H-2D^b (*l*). Note particularly the nonreciprocity of the results in *e* and *f* with those in *i* and *j*.

(*m–p*) P815 cells (K^dD^d) 3 hr after infection with VSV Indiana. After the VSV-G protein was patched or capped by fluorescein-labeled indirect antibody treatment (*m* and *o*), the cells were fixed and then stained by rhodamine-labeled indirect antibody for the H-2K^d antigen (*n*) or the H-2D^d antigen (*p*). Neither the H-2K^d antigen (*n*) nor the H-2D^d antigen (*p*) had significantly co-redistributed with the VSV-G protein (*m* and *o*).

(*q–x*) Controls. (*q–t*) EL4 cells 3 hr after infection with VSV New Jersey (instead of VSV Indiana). The H-2K^b antigen (*r*) or the H-2D^b antigen (*t*) was first capped by rhodamine-labeled indirect antibody treatment, and then the cells were fixed and stained by fluorescein-labeled indirect antibody for VSV Indiana G-protein (*q* and *s*). No staining was observed of the non-crossreactive VSV-G-protein (*q* and *s*). EL4 cells 3 hr after infection with VSV Indiana were treated with fluorescein-labeled indirect antibody for VSV Indiana (*u*); then the cells were fixed and treated with rhodamine-labeled indirect antibody to the H-2K^d antigen (*v*); no staining was observed in *v*. Similar negative controls were obtained with the appropriate cells and the other anti-H-2 antisera. MC57G cells 3 hr after infection with VSV Indiana were first fixed and stained for VSV-G protein (*w*) and for H-2K^b antigen (*x*), showing the initially uniform distributions of these two surface antigens.

H-2D restriction in the cytotoxic lysis of VSV-infected target cells was achieved by blocking the lytic reaction in a syngeneic effector cell/target cell system by using different anti-H-2 antibodies (19). It was found (Table 2) that the anti-H-2K^b antiserum was more effective than the anti-H-2D^b antiserum in blocking the cytotoxic lysis of 2-hr VSV-infected MC57G target cells (K^bD^b) by C57BL/6 effector cells (K^bD^b). The two antisera had comparable titers toward their respective H-2 antigens as determined by complement-mediated cytotoxicity assays on both MC57G and EL4 cells and by radioimmunolabeling assays similar to those given in Table 1 (not shown). Although inhibition was greater with the anti-H-2K^b antiserum than with the anti-H-2D^b antiserum, the two antisera showed a synergistic effect when mixed: when diluted 1:5, each antiserum alone had only a small or marginal effect, but when these dilutions were mixed 1:1, the inhibition was nearly complete. As controls, anti-H-2K^d antisera and mixtures of anti-H-2K^d plus anti-H-2D^d antisera were ineffective inhibitors of this H-2^b-restricted cytotoxic reaction.

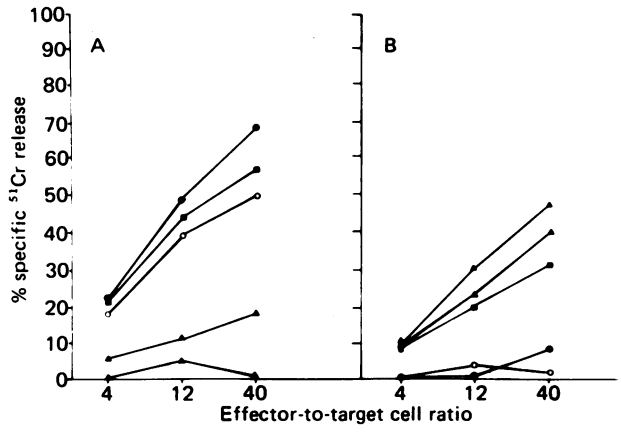

FIG. 2. Cytotoxic lysis (as measured by ^{51}Cr release) of VSV-infected MC57G (K^bD^b) (*A*) and VSV-infected P815 (K^dD^d) (*B*) target cells, by effector cells from different inbred mouse strains: ●, C57BL/6 (K^bD^b); ○, BALB/B (K^bD^b); ▲, BALB/c (K^dD^d); ■, B10.A(5R) (K^bD^d); △ B10.HTG (K^dD^b).

DISCUSSION

Most of the integral proteins of membranes appear to exist as individual molecules that are independently mobile in the plane of the membrane (20). Evidence supporting this view has come from experiments on the antibody-induced capping of specific integral proteins, which generally have shown that the capping of component A does not perceptibly change the random distribution of component B in the membrane (cf. ref. 21). The relatively infrequent cases in which, on the contrary, the antibody-induced capping of component A results in the co-capping of the antigenically unrelated component B are generally thought to reflect the existence of a stable molecular complex between A and B. Thus, a number of previous studies have provided evidence that an H-2 antigen and some particular antigen A may co-cap (7–10). For example, with murine leukemia cells of the *H-2*^{b} haplotype transformed by Rauscher murine leukemia virus, the antibody-induced patching and capping of the *H-2D*^{b} antigen caused a co-patching and co-capping of the gp70 cell surface antigens associated with the virus (9). Such experiments sometimes have been taken to mean that the H-2 and viral antigen components form a molecular complex in the membrane and therefore as supporting the "altered self" model of cytotoxic T-cell recognition. On the other hand, the male H-Y antigen, which also serves as a target for H-2-restricted cytotoxic killing (6), did not co-cap extensively with H-2 molecules (11). In this paper, we have shown that, although the capping of the VSV-G protein is accompanied by co-capping of the H-2K^b antigen, the effect is not reciprocal despite the fact that the two antigen molecules are present in comparable numbers in the membrane (Table 1). The nonreciprocity is inconsistent with the formation of a stable molecular complex between the H-2K^b and VSV-G proteins. In another study, Bourguignon *et al.* (22) showed that the antibody-induced capping of the T-200 antigen on mouse thymoma cells was accompanied by the apparent co-capping of H-2, TL, and Thy-1 antigens, although the latter three components are normally molecularly independent of one another in membranes (23).

Table 2. Anti-H-2 antibody inhibition of cytotoxic lysis of VSV-infected MC57G target cells by C57BL/6 effector cells

Antiserum, dilution				%
Anti-H-2K^b	Anti-H-2D^b	Anti-H-2K^d	Anti-H-2D^d	inhibition*
Undil.	—	—	—	93
1:5	—	—	—	25
—	Undil.	—	—	33
—	1:5	—	—	4
1:5	1:5	—	—	97
—	—	Undil.	Undil.	16
—	—	1:5	1:5	0

* Corrected for small inhibition by normal mouse serum.

It seems likely in such instances that it is the antibody-induced clustering of a particular integral protein such as VSV-G or T-200 that causes an association of these clusters with certain other previously independent integral proteins in the plane of the membrane. We propose that the co-capping that results from such cluster-induced associations be designated by the term *syn*-capping. The molecular mechanisms that may be involved in *syn*-capping are not clear at present. One possibility is that a small cluster of component A molecules in a membrane may exhibit an enhanced affinity for a molecule of component B over that of individual A and B molecules for one another, a situation that may be roughly analogous to that which occurs in the complement–Ig system—clustered Ig sites have a higher affinity than do single Ig sites for C1q (24). Another possibility may be that the clustering of component A may induce a transmembrane interaction of the clusters with certain cytoskeletal proteins (15, 25–29); the cytoskeleton may then somehow participate in the interaction of clusters of component A with component B.

On occasion, it may be difficult to determine whether a given instance of co-capping is actually *syn*-capping. The finding that the co-capping of components A and B is reciprocal (e.g., ref. 9) does not rule out the possibility that A and B are molecularly independent but that *syn*-capping is occurring in both directions. On the other hand, the apparently nonreciprocal co-capping of components A and B is not a sufficient condition to establish that *syn*-capping is involved. If the numbers of B molecules considerably exceeded those of A and a large excess of free B were present along with stable A–B complexes, A would appear to co-cap with B but not *vice versa*. The absence of quantitative information such as is given in Table 1 therefore makes it unclear whether *syn*-capping is involved in several such cases[§] (cf. refs. 10, 31, and 32).

Our immediate interest in the phenomenon of *syn*-capping is its possible relevance to the molecular mechanisms involved in cytotoxic T-cell recognition and its H-2 restriction. Bourguignon *et al.* (22) presented an outline of a dual receptor mechanism for such recognition, in which a *syn*-capping process plays a central role. The mechanism involves the following elements. (*i*) The cytotoxic T cell is assumed to contain in its membrane two molecularly independent recognition molecules, a clonally segregated one for an H-2 antigen (anti-H) and one specific for the immunizing antigen A (anti-A). The target cell membrane contains molecularly independent A and H-2 molecules. (*ii*) Upon contact of a cytotoxic T cell and a target cell, anti-A molecules in the former and A molecules in the latter bind to one another. Once a small number of anti-A–A bonds are simultaneously formed so as to sufficiently stabilize the cell–cell contact, the lateral diffusion of anti-A and A molecules in their respective membranes then allows the rapid formation of large numbers of anti-A–A bonds in the region of

§ A phenomenon related to *syn*-capping may provide the explanation for the observations by several investigators (18, 30) that H-2 antigens are found associated with isolated mature particles of certain membrane-bound viruses. Such viruses mature by budding out of the host cell membrane. If a part of this process involves the collection of the viral protein molecules into clusters in the host cell membrane, such clusters may have an affinity for the molecules of one or more H-2 components in the membrane, and the H-2 may thus become incorporated into the ultimately released virus particles.

cell–cell contact. In effect, the A and anti-A molecules mutually patch or cap one another into the contact region. (*iii*) Such patching or capping of A molecules in the target cell membrane causes an association or *syn*-capping of one or more H-2 antigens with clusters of A molecules. (*iv*) This concentration of H-2 molecules into the region of cell–cell contact results in the binding and redistribution of anti-H molecules in the membrane of the T cell into the contact region.¶ The net effect of steps *ii–iv* is to create a transiently stable linkage between the T cell and the target cell which is defined by the sum of intercell bonds between anti-A and A and anti-H and H-2 molecules, a linkage that might be much less stable in the absence of the anti-A–A interaction. (*v*) Some killing event is assumed to be mediated by the anti-H–H binding that is thus stabilized in the contact region between the two cells.

This mechanism of the cytotoxic T-cell killing, and its extension to the induction of the cytotoxic response, will be considered in greater detail elsewhere. We do not exclude the possibility that K- or D-associated T-cell low responsiveness to virus may be caused by a defect of the T-cell repertoire (3). Here we note that an important element of the mechanism is the proposal that, for efficient cytotoxic killing, T-cell-induced clustering (patching or capping) of certain antigens A in target cell membranes induces a *syn*-patching or *syn*-capping of one or more H-2 antigens with A clusters. That a *syn*-capping of H-2 antigens can occur with viral antigens is supported by our present results. Furthermore, that such *syn*-capping might indeed be functionally important for cytotoxic T-cell interactions is suggested by the striking correlation between H-2 *syn*-capping efficiency and cytotoxic T-cell killing efficiency. Cytotoxic T-cell-mediated lysis of VSV-infected target cells is severalfold more efficient when directed against the H-2K^b than the H-2D^b antigen (Fig. 2*A*), and anti-H-2K^b antibodies are severalfold more effective than anti-H-2D^b antibodies in inhibiting the lytic reaction (Table 2). This apparent efficiency of killing as measured here is a composite of the efficiency of the induction of cytotoxic T cells (killer cell number) and the average rate with which a single killer cell lyses its target (intrinsic killer cell efficiency). The present measurements do not separate these two factors. These results correlate with the finding that there occurs a more extensive *syn*-capping of H-2K^b than H-2D^b with antibody-induced clusters of VSV-G protein on target cells. Likewise, the lesser efficiency of cytotoxic T-cell lysis of VSV-infected cells of the *H-2^d* compared to the *H-2^b* haplotype (Fig. 2*B*) and the similar efficiency of lysis directed to the H-2K^d and H-2D^d antigens are correlated with the observations (Fig. 1 *m–p*) that the H-2K^d and H-2D^d antigens are both much less extensively *syn*-capped with VSV-G protein on P815 cells than is H-2K^b with VSV-G on either MC57G or EL4 cells.

It should be pointed out that the mechanism outlined above does not require a complete or even an extensive *syn*-capping of a particular H-2 antigen with a target antigen A in order to produce the cytotoxic interaction. A small degree of H-2 *syn*-capping may be sufficient.¶ There is therefore no necessary contradiction in the findings that specific cytotoxic killing of VSV-infected target cells occurs through the mediation of the H-2D^b, H-2K^d, and H-2D^d antigens despite their lack of extensive *syn*-capping with antibody-induced clusters of VSV-G protein.

It clearly would be desirable to examine in similar detail the cytotoxic T-cell responses to various antigens other than VSV, such as other viruses and minor transplantation antigens, which show H-2 restrictions that are different from those found with VSV to determine whether H-2 *syn*-capping occurs with these antigens and whether the correlation between the efficiency of *syn*-capping and H-2-directed cytotoxic lysis applies to these antigens as well as to VSV.

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¶ In principle, simply the mutual capping of anti-A and A molecules generating a region of cell–cell contact may itself be sufficient (without requiring a *syn*-capping event) to favor thermodynamically the redistribution and bond formation between H and anti-H molecules into the same contact region. Such a process, however, could not readily account for *preferential* K- or D-end H-2 restriction in cytotoxicity reactions.