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SIGNAL TRANSDUCTION THROUGH CLASS I MHC BY A MONOCLONAL ANTIBODY THAT DETECTS MULTIPLE MURINE AND HUMAN CLASS I MOLECULES[1]

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We have generated a hamster anti-mouse class I reactive mAb that is capable of activating T cells in the presence of the cofactor PMA, as assayed by both IFN-γ production and cellular proliferation. This mAb detects an epitope present on the majority of murine class I molecules, with the known exceptions of H-2K^k and H-2K^q, and is therefore not β_2-microglobulin-specific. It also recognizes multiple human class I molecules. The epitope recognized by this antibody maps to the class I α_1 domain. The activation properties of this mAb are not mediated exclusively through the glycosylphosphatidylinositol-linked Qa-2 molecule, as the antibody activates spleen cells from Qa-2-negative strains. Although class I molecules are not usually considered as activation Ag, these data demonstrate their potential for involvement in signal transduction.

Under physiologic conditions, T cells are activated after interaction of peptide Ag presented by class I or II MHC molecules. This effect can be mimicked in many respects by antibodies directed to a number of cell surface Ag, which fall broadly into two categories. The first category comprises transmembrane proteins and includes CD2 (1), CD3 (2, 3), and CD28 (4, 5). These molecules typically require a cytoplasmic domain in order to function in signal transduction (6). A second category of receptors, including Thy-1 and members of the Ly-6 complex, are anchored to the cell membrane via GPI[5] linkages (7, 8). The class I MHC Qa-2 molecule is also GPI-linked (9), and antibodies directed toward it are mitogenic for T cells (10). It is the GPI anchor that is critical for activation via this molecule (11).

The class I molecules, H-2K, H-2D, and H-2L, are integral cell surface glycoproteins expressed in association with β_2-microglobulin. They are not typically thought of as activation Ag, as antibodies to these class I MHC molecules are not normally mitogenic for T cells. However, this view has recently been challenged by the demonstration that cross-linking of HLA class I molecules can enhance stimulation by mitogenic antibodies to other cell surface molecules, and can induce an intracellular increase in (Ca^{2+}), and proliferation of CD4$^+$ T cells in the presence of PMA (12, 13). Therefore, it has been postulated that signal transduction may occur via classic class I molecules and that these molecules may play a role in modulating T cell activation.

In the course of generating mAb against molecules involved in signal transduction in murine T cells, we generated an activating antibody that detected class I molecules. The studies presented here indicate a potential role for classic class I MHC molecules in signal transduction in addition to that attributable to Qa-2.

MATERIALS AND METHODS

Mice. Mice were purchased from The Jackson Laboratory (Bar Harbor, ME), or the Frederick Animal Facility (Frederick, MD). Armenian hamsters were obtained from Toxicon (Boston, MA). Animals were maintained in a specific pathogen-free barrier facility at the University of Chicago in accordance with guidelines of the Animal Resource Center.

mAb. The mAb UC3-10H3/3 was derived from the fusion of immune spleen cells from an Armenian hamster with the myeloma SP2/0. The hamster was immunized s.c. in complete Freund's adjuvant, and then i.p. in incomplete Freund's adjuvant four times over an 8-wk period with 10^7 BALB/c *nu/nu*-derived G8 TCR-γδ CTL clone (14, 15). After a 6-week interval, the animal was boosted i.v. Spleen cells from this animal were fused 3 days later. The fusion was screened by antibody-induced proliferation as described below, and cloned by the limiting dilution method.

Other mAb used in this study together with their specificity include anti-CD3, 145-2C11 (3); unknown, 145-10D8; anti-K^b/Qa-2/D^b/K^d/H-2^q, 20-8-4 (16, 17); and other class I reactive mAb: anti-D^b/L^d/D^q/L^q, 28-14-8; anti-K^b, 5F1; anti-D^d, 34-2-12; anti-K^k, 36-7-5; anti-K^k/D^k/K^b/H-2^q, 3-83; anti-K^k/H-2^q, 11-4-1; anti-D^k/K^d/H-2^q, 15-5-5; anti-H-2^k, 15-1-5; anti-D^q/L^q/L^d, 30-5-7 (18); and mouse anti-hamster Ig, AH6 (19). Specificities listed in the text are restricted to reactivity in the appropriate haplotype described.

Cell lines. The BALB/c *nu/nu* derived G8 TCR-γδ CTL clone has been described (14, 15). The AKR-derived R1.1 thymoma and R1.E β_2-microglobulin negative variant have also been described (20). The human C1R cells and the HLA-A3, -B7, and Bw57.2 C1R-transfectants were provided by Dr. P. Creswell (21). The IT22.6 H-2^q-expressing fibroblast cell lines transfected with exon-shuffled H-2K gene constructs were obtained from Dr. B. Arnold (German Cancer Research Center, Heidelberg) (22).

Fluorocytometric analysis. Fluorocytometric analysis was performed after staining single cell suspensions sequentially with antibody and FITC-coupled second step reagent (FITC-conjugated protein A or mouse anti-hamster mAb (AH6)) as described (23) using an EPICS 753 flow cytometer.

Biochemistry. Biochemistry was performed as described previously (24). Cells (0.5 to 5 × 10^7) were washed three times in PBS and labeled with ^{125}I using the lactoperoxidase catalyzed iodination procedure as described (25). Cells were then lysed in 0.5% NP-40, pH 8,

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[5] Abbreviation used in this paper: GPI, glycosylphosphatidylinositol.

centrifuged to remove nuclei, and pre-cleared by overnight incubation at 4°C with hyperimmune rabbit anti-KLH antiserum and protein-A agarose (BRL). In some experiments, preclearing of specific class I molecules was performed in conjunction with this step by including 25 μl of ascites and an additional 100 μl of pA agarose. Precipitates were electrophoresed under reducing conditions in 15% SDS-PAGE gels. Gels were autoradiographed at −70°C using Kodak XAR-5 film and Lightning-plus intensifying screens.

Antibody-induced IFN-γ production. Costar 96-well microtiter wells were coated with 100 μl of purified mAb (10 μg/ml) and washed. Cultures were established with 1×10^5 G8 cells and 10 ng/ml of PMA in a total of 200 μl. After incubation for 24 to 48 h at 37°C, the supernatant was harvested, and IFN-γ production was determined using an ELISA (26), as with modifications as described (27).

Antibody-induced proliferation. Cultures were established in a total of 200 μl in 96-well Costar microtiter wells, containing a final concentration of 25% of mAb culture supernatant, 4×10^5 lymph node cells, and 10 ng/ml of PMA. After incubation for 48 h at 37°C, 1 μCi of [^{3}H]thymidine (Amersham) was added to each well and the cells cultured for an additional 16 to 20 h. The wells were harvested, and incorporated radiolabel was assessed using a liquid scintillation counter.

RESULTS

The UC3-10H3/3 mAb recognizes an MHC-encoded determinant expressed by the majority of lymphoid and hemopoietic cells. In an effort to define novel Ag involved in T cell activation responses, mAb were produced by immunizing a hamster with a BALB/c TCR-γδ T cell clone, G8. Antibodies were screened for their ability to induce G8 activation, and those of interest were further characterized. One such activating antibody, UC3-10H3, consistently induced a modest IFN-γ response in G8 cells (Table I) and a greater than four-fold proliferative response by B10.D2 spleen cell cultures, when PMA was used as a cofactor (Table I). Both responses were generated with the anti-TCR mAb in the presence and absence of PMA, whereas no proliferative response was obtained with the class I-specific mAb, 15-5-5, even in the presence of PMA.

By flow cytometric analysis, the mAb stained the G8 cell line (H-2^d) and other T cell clones strongly (data not shown). Further analysis revealed that the determinant recognized by this antibody was not restricted to T cells, as it also stained >95% of spleen, lymph node, bone marrow, and thymus cells from B10.D2 (Fig. 1, *A* to *B*). Thus, the antibody reacts with most hematopoietic cells from H-2^d animals.

Cytometric analysis revealed that as well as being expressed on B10.D2 spleen cells, the determinant was also expressed on C3H/HeN (H-2^k) (Fig. 2*B*) and C57BL/10 (H-2^b) (Fig. 2*D*). Such extensive cross-reactivity of the UC3-10H3/3 mAb on murine cells from different strains led us to investigate if the mAb reacted with an antigenic determinant expressed on human cells. Flow-cytometric analysis of PBL obtained from two unrelated individuals indicated that the Ag was expressed by both of them (Fig. 2, *C* and *E*). Therefore, the epitope recognized by this antibody was very highly conserved both within and between species. However, the mAb does not detect β_2-microglobulin (discussed below).

UC3-10H3/3 recognizes a determinant expressed on class I molecules. The molecular nature of the Ag recognized by this antibody was established by cytometric and biochemical analyses. Immunoprecipitation from C57BL/10 spleen cells with the UC3-10H3 antibody revealed 45- and 40-kDa molecules (Fig. 3, *lane 1*), which co-migrated with class I molecules precipitated by the anti-D^b mAb, 28-14-8, (Fig. 3, *lane 3*) and the anti-K^b/D^b/Qa-2 mAb, 20-8-4 (Fig. 3, *lane 4*). The high molecular mass material co-migrated with D^b (Fig. 3, *lane 3*) and K^b (Fig. 3, *lane 4*) so it was not possible to determine if UC3-10H3 reacted with one or both of these molecules. However, the 40-kDa material co-migrated with 40-kDa protein precipitated by 20-8-4 (Fig. 3, *lane 4*), known to be

TABLE I

Proliferation and IFN-γ induction by soluble UC3-10H3 mAb[a]

Antibody	PMA	IFN-γ (U/mL)	Proliferation (cpm × 10^{-3})
NIL	−	<10	1.2 ± 0.08
	+	36	2.4 ± 0.3
Anti-class I (UC3-10H3)	−	27	3.2 ± 0.4
	+	112	11.1 ± 2.8
Anti-CD3 (145-2C11)	−	114	53.0 ± 2.1
	+	175	95.9 ± 2.2
Anti-H-2^k (15-1-5)	−	NT	0.9 ± 0.06
	+	NT	2.5 ± 0.1

[a] The IFN-γ assay was performed using purified antibody coated to plastic to stimulate G8 cells, whereas the proliferation response of B10.D2 spleen cells was measured after stimulation with soluble antibody. Both assays were performed as described in *Materials and Methods*.

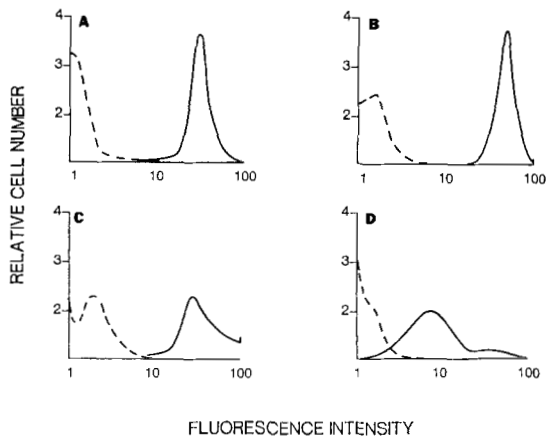

Figure 1. Tissue distribution of the UC3-10H3 epitope. FACS analysis of B10.D2 spleen (*A*), lymph node (*B*), bone marrow (*C*), and thymus stained with UC3-10H3 and mouse anti-hamster AH6-FITC (——) or AH6-FITC alone (– – –).

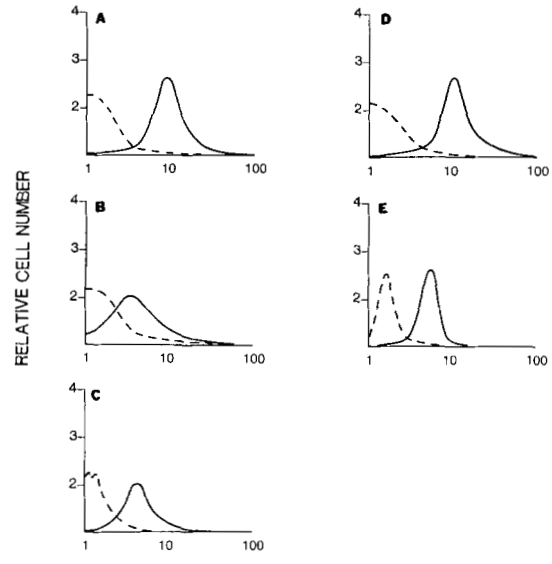

Figure 2. Strain and species distribution of the UC3-10H3 epitope. FACS analysis of spleen cells from B10.D2 (*A*), C3H (*B*) and C57BL/10 (*D*), and human PBL from individual JE (*C*) and BH (E) stained with UC3-10H3 and protein A FITC (——) or pA FITC alone (– – –).

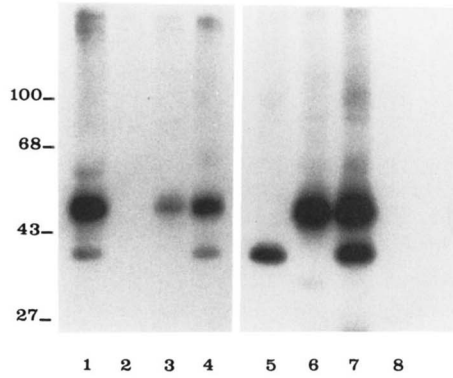

Figure 3. UC3-10H3 detects D^b and Qa-2. Immunoprecipitation of ^{125}I-labeled C57BL/10 ($K^bD^bQa\text{-}2^a$) (*lanes 1* to *4*) and B10.A(2R) ($K^kD^bQa\text{-}2^a$) (*lanes 5* to *8*) spleen cell lysates using UC3-10H3 (*lanes 1* and *7*); control hamster antibody, 145-10D8 (*lane 2*); anti-D^b, 28-14-8 (*lanes 3* and *6*); anti-K^b/D^b/Qa-2, 20-8-4 (*lanes 4* and *5*); and anti-D^d 34-2-12 (*lane 8*).

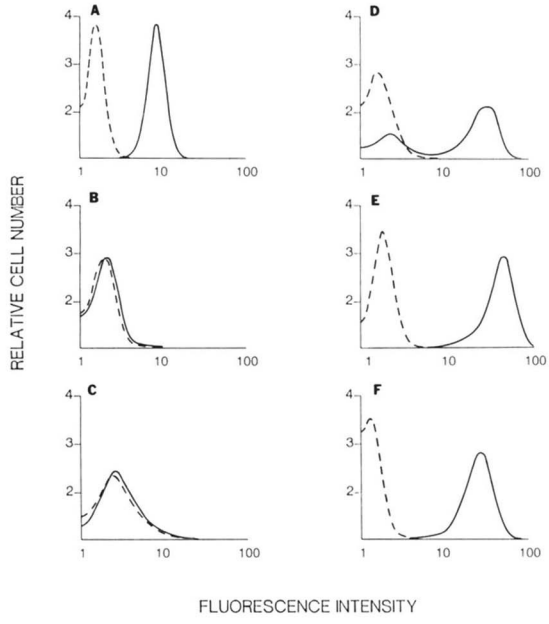

Figure 4. UC3-10H3 detects determinants expressed on murine and human class I molecules. FACS analysis of the murine cell lines R1.1 (*A*) and the β_2-microglobulin negative variant R1.E (*B*), and the human class I-negative variant C1R (*C*), and C1R cells transfected with HLA-A3 (*D*), HLA-B7 (*E*), and HLA-Bw57.2 (*F*). Cells were stained with UC3-10H3 and protein A-FITC (——) or FITC alone (– – –).

Qa-2 (17), suggesting Qa-2 reactivity of UC3-10H3. This 40-kDa protein was precipitated from the B6.K2 strain by UC3-10H3, but was not precipitated from the Qa-2 negative congenic strain B6.K2 (data not shown), again suggesting reactivity with the Qa-2 molecule.

Class I-like reactivity of the mAb was confirmed by cytometric analysis of the H-2^k R1.1 thymoma, which was stained by the UC3-10H3/3 mAb, whereas the β_2-microglobulin variant R1.E was not stained (Fig. 4, *A* and *B*). To determine if the reactivity to human cells was directed against a determinant expressed on class I molecule(s), HLA-transfectant cells were analyzed. The class I negative human cell line C1R was not stained (Fig. 4*C*). However, C1R cells transfected with HLA-A3, HLA-B7, and HLA-Bw57.2 stained positively with the antibody (Fig. 4, *D* and *F*). Thus, this antibody reacts with determinants expressed on murine and human class I molecules.

As discussed, this antibody clearly detected an epitope present on class I molecules on spleen cells from many haplotypes, including B10.D2 (H-2^d), C3H (H-2^k), and C57BL/10 (H-2^b) (Fig. 2). Additional analysis revealed that the mAb reacted with spleen cells from strains including B10.M (H-2^f), B10.Q (H-2^q), and B10.S (H-2^s) B10.P (H-2^p), A.TH (H-2^{t2}), and A.AL (H-2^{a1}) (data not shown). In fact, the mAb has reacted with a determinant expressed on all strains examined to date.

Although UC3-10H3 detects an epitope expressed on all strains investigated, the staining of the H-2^k strains C3H and B10.BR was much weaker (Fig. 2, Table II), whereas the staining of B10.A and B10.A(2R) was intermediate (Table II). This differential staining pattern combined with the immunoprecipitation data suggested that the mAb recognized a polymorphic determinant(s) expressed on subsets of murine class I molecules encoded by multiple loci.

Further biochemical analysis was undertaken to determine which class I molecules were recognized by UC3-10H3 mAb. Reactivity with D^k molecules was established by immunoprecipitation from C3H-derived L cells. UC3-10H3 (Fig. 5, *lane 3*) precipitated a protein that co-migrated with D^k (Fig. 5, *lane 1*), but that was larger than K^k (Fig. 5, *lane 4*). Reactivity to D^k was formally demonstrated by preclearing experiments. When L cell lysates were precleared of D^k molecules before immunoprecipitation with UC3-10H3, class I reactivity of this mAb was abolished (Fig. 5, *lanes 5* to *8*). Thus, D^k appears to be the only molecule detected by UC3-10H3 on L cells. Furthermore, since D^k but not K^k is recognized by this mAb,

TABLE II

Strain distribution pattern of UC3-10H3

Strain	MHC Haplotype K/D/Qa-2	Fluorescence Ratio[a]	mAb Reactivity	MHC Class I Molecules Detected
C57BL/10	b b a	19.0	+++	K, D, Qa-2
B10.BR	k k b	4.5	+	D
B10.A(2R)	k b a	12.4	++	D, Qa-2
B6.K1	b b b	16.7	++	K, D
B6.K2	b b a	20.2	+++	K, D, Qa-2

[a] FACS analysis of spleen cell populations, in which the ratio of fluorescence is defined as:

$\log_{10}$ *mean peak of fluorescence UC3-10H3+protein* A^{FITC} – *mean peak of fluorescence* pA^{FITC} *alone*

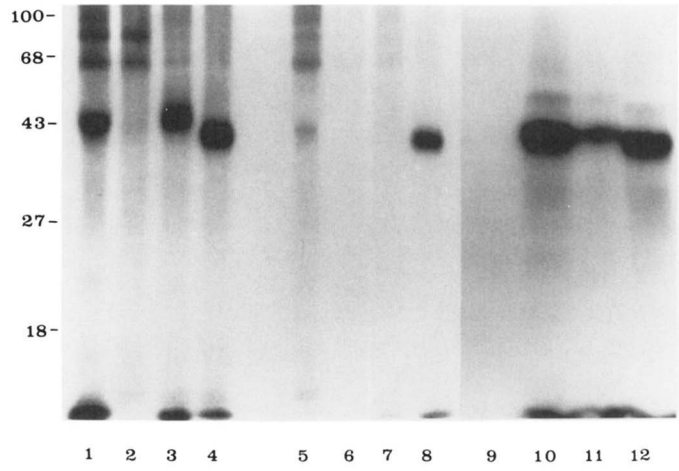

Figure 5. 10H3 detects D^k and K^b. Immunoprecipitation of class I molecules from ^{125}I-labeled L cell lysates (*lanes 1* to *4*) or lysates precleared for D^k molecules with the anti-D^k antibody 15-5-5 (*lanes 5* to *12*) derived from L cells (*lanes 5* to *8*) and L cells transfected with K^b and (*lanes 9* to *12*). Antibodies used for immunoprecipitation were anti-D^k, 15-5-5 (*lanes 1, 5,* and *9*); anti-K^b, 20-8-4 (*lanes 2, 6,* and *10*); UC3-10H3 (*lanes 3, 7,* and *11*); and anti-K^k, 36-7-5 (*lanes 4, 8,* and *12*).

UC3-10H3 is not specific for β_2-microglobulin.

Reactivity with a determinant expressed on K^b was demonstrated by analysis of L cells transfected with K^b. Since we previously showed that the only detectable protein precipitated by UC3-10H3 from L cells was D^k, L cells transfected with K^b were radiolabeled, and the lysates precleared for D^k using mAb 15-5-5. Under these conditions, reactivity of UC3-10H3 to K^b was clearly demonstrated (Fig. 5, *lane 11*).

To characterize which additional class I molecules could be detected by UC3-10H3, biochemical studies were performed using the B10.A(2R) strain (K^k D^b), since UC3-10H3 does not react with K^k. UC3-10H3 precipitated two proteins from spleen cells of this strain. The smaller Qa-2 molecule was again precipitated by UC3-10H3 antibody (Fig. 3, *lane 7*) and by 20-8-4 (Fig. 3, *lane 5*). In addition, a 45-kDa molecule was precipitated that co-migrated with the major species precipitated by the anti-D^b reactive mAb 28-14-8 (Fig. 3, *lane 6*). Therefore, UC3-10H3 probably recognizes a determinant expressed on D^b.

Therefore, the biochemical and cytofluorometric analysis taken together suggest that the high staining of UC3-10H3 on C57BL/10 is due to recognition of a determinant expressed on K^b, D^b, and Qa-2. The intermediate staining of B10.A(2R) is accounted for by detection of a determinant expressed on D^b and Qa-2, whereas the D^k molecule is the only known molecule to express the UC3-10H3 determinant in the weakly staining C3H and B10.BR strains (Table II). Thus, differential staining patterns can be attributed to the number of class I molecules detected.

The antibody is directed to an epitope determined by the first domain of class I molecules. The domain of the class I molecules responsible for binding of the mAb was established by biochemical analysis of hybrid K^d/K^k molecules. The H-2^q IT22.6 cell line, which had been transfected with exon-shuffled H-2K genes and which expressed combinations of α_1 and α_2 K^k and K^d domains with α_3 transmembrane and intracytoplasmic portions derived from K^k, was analyzed. Initial biochemical data established that UC3-10H3 did not react with K^q but did react with D^q and/or L^q (data not shown). Therefore, analysis of the exon-shuffled transfectants was undertaken after preclearing with D^q/L^q specific mAb 28-14-8. After preclearing for D^q/L^q, UC3-10H3 reacted with transfectant IC33 (α_1 K^d, α_2 K^d) (Fig. 6). UC3-10H3 did not react with K molecules expressed by IC35 (Fig. 6), which contained K^d in the second domain only (α_1 K^k, α_2 K^d), implying the reactivity of UC3-10H3 for IC33 required the first, not the second domain. This was confirmed by the reactivity of the mAb to IC31 (α_1 K^d, α_2 K^k) (Fig. 6), which contained only the α_1 domain of D^d, thus localizing the binding requirement to the first domain.

Antibody-induced proliferation in strains that lack Qa-2 expression. Qa-2 is linked to the cellular membrane via a GPI linkage and triggering through this molecule has been demonstrated to result in T cell activation (10, 11). Since UC3-10H3 recognizes Qa-2 or a Qa-2-like molecule, and induced modest proliferative responses in spleen cell cultures, we wished to determine whether this activity was primarily mediated through the Qa-2 molecule. Therefore, the activating properties of UC3-10H3 were examined using spleen cells from different strains of mice, including some that lacked Qa-2 expression. As demonstrated, a two- to fivefold proliferative response was observed in the Qa-2^+ strains C57BL/10, C57BL/6, and B6.K2, as well as in the Qa-2^- strains B10.BR and B6.K1 (Table III). These results demonstrate that the proliferative response to UC3-10H3 does not depend on the presence of the Qa-2 molecule, although we cannot discount that signal transduction could be occurring via this molecule in strains in which it is expressed.

DISCUSSION

This study was undertaken to identify novel activation-associated molecules on T lymphocytes. One mAb, UC3-10H3, was selected for additional study based on its ability to induce IFN-γ production and proliferative responses in T lymphocytes. It is specific for class I molecules, but displayed extensive cross-reactivity on every haplotype examined. Serologic and biochemical data suggest that in the H-2^k haplotype, this antibody recognizes H-2D, but not H-2K molecules. Therefore, UC3-10H3 is clearly not β_2-microglobulin specific. In the H-2^b haplotype, H-2K, H-2D, and Qa-2 are detected. Furthermore, fluorescence analysis revealed differential staining of B10.D2 and the B10.A strains, implying reactivity in the H-2^d haplotype with H-2K (Table I). It is unknown if the determinant recognized by this antibody expressed on H-$2D^d$ and/or H-$2L^d$, but reactivity of at least one of these molecules is indicated by the similar level of staining of B10.D2 and C57BL/10 (Table I). In addition, there is evidence that Tla is not precipitated by this antibody, as determined by immunoprecipitation of L cells transfected with $T3^b$ (data not shown). Qa-1 reactivity has not been determined. Finally, this antibody reacted with HLA-A and HLA-B class I molecules expressed on PBL and transfected cell lines, and therefore recognizes an epitope that is highly conserved within and between species.

The epitope of the H-2K molecule bound by the antibody is dependent on amino acid sequences in the first domain. We have been unable to precisely locate the epitope, inasmuch as the mAb does not react with the α_1-domain peptides tested to date. However, based on available sequence data, there are three likely regions for binding of the antibody within the first domain, which are conserved between $K^b/K^d/D^b/D^d$ but not conserved in K^k. These are the regions around amino acid numbers 19, 49, and 55, which are also very highly conserved in human class I molecules. Based on the x-ray crystal structure of HLA-A2 (28), all three amino acids are relatively exposed. However, it is possible that the binding site lies outside of any one of these regions, and is influenced by conformational restrictions imparted by another region within the first domain, or by other parts of the molecule with which it may interact.

Due to the highly cross-reactive specificity of UC3-10H3, it was necessary to dissect which molecule was participating in signal transduction in T lymphocytes. The UC3-10H3 mAb reacts with the Qa-2 molecule, which is a GPI-linked protein (9), and this anchor has recently been demonstrated to be a requirement for Qa-2-mediated-T cell activation (11). The anti-Qa-2 mAb generally give proliferative responses of up to 100-fold above background in spleen cell cultures, in the presence of the co-factor PMA and anti-Ig mediated cross-linking (10, 11). Furthermore, only α_3-domain reactive mAb had the potential to activate T cells (10). Two pieces of information suggest that UC3-10H3 does not activate via the

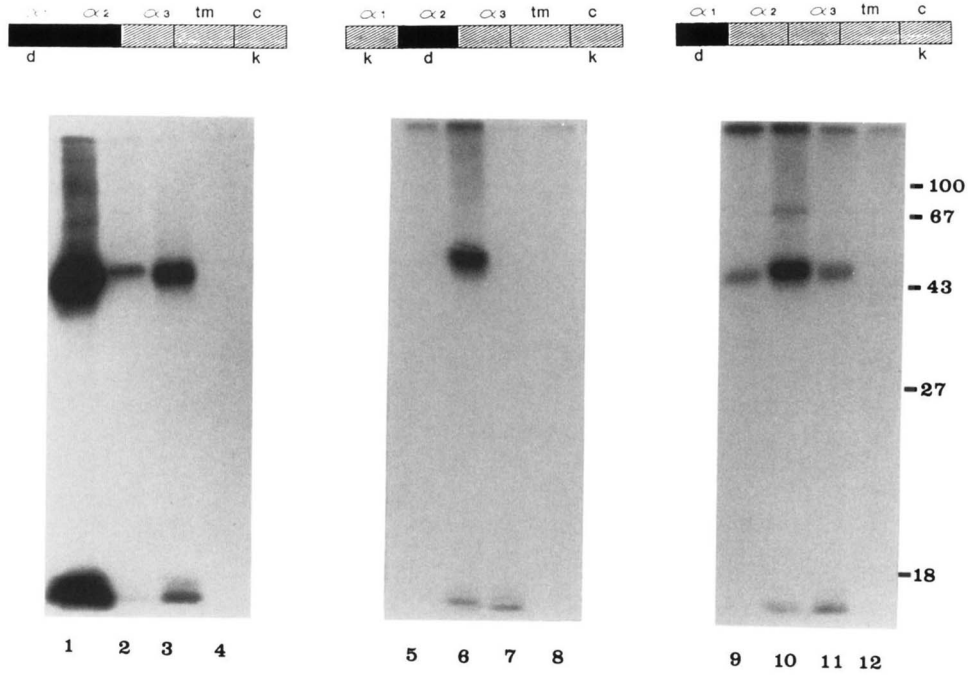

Figure 6. α_1-Domain of K^d is necessary and sufficient for UC3-10H3 binding. Immunoprecipitation analysis of H-2^q IT22.6 transfectants IC33 (*lanes 1* to *4*), IC35 (*lanes 5* to *8*), and IC31 (*lanes 9* to *12*), containing exon-shuffled D^k/K^k molecules, where the origin of the exons is indicated as being from either K^d (*shaded*) or K^k (*striped*). Before immunoprecipitation with the immunoprecipitating antibodies, lysates were precleared of D^q molecules with 30-5-7 and 28-14-8 cross-reactive antibodies as described in *Materials and Methods*. Antibodies used were anti-K^d/H-2^q, 20-8-4 (*lanes 1, 5,* and *9*); anti-K^k, 3-83 and 11-4-1 (*lanes 2, 6,* and *10*); UC3-10H3 (*lanes 3, 7,* and *11*); or anti-D^q, 30-5-7 (*lanes 4, 8,* and *12*).

TABLE III

Proliferative responses induced by antibody in strains that lack Qa-2 expression[a]

	Strain	mAb	Proliferation (cpm × 10^{-3})
Expt. I	C57BL/10		8.9 ± 0.1
		145-2C11	61.3 ± 8.0
		UC3-10H3	19.9 ± 3.3
	B10.BR		8.8 ± 1.2
		145-2C11	56.2 ± 8.0
		UC3-10H3	18.5 ± 1.6
Expt. II	C57BL/6		3.7 ± 0.4
		145-2C11	46.4 ± 3.4
		UC3-10H3	11.2 ± 1.7
	B6.K1		3.6 ± 0.1
		145-2C11	43.9 ± 1.0
		UC3-10H3	10.0 ± 0.5
	B6.K2		3.3 ± 1.2
		145-2C11	55.7 ± 4.3
		UC3-10H3	15.0 ± 1.4

[a] PMA (10 ng/ml) was included in all cultures.

Qa-2 molecule. 1) The activation effect is observed in strains that lack expression of the Qa-2 molecule. In fact, since the only known molecule recognized in the C3H strain is D^k, it is possible that activation/signal transduction can occur via interaction with this molecule. 2) Proliferative responses are not enhanced upon cross-linking. Thus, we have reason to believe that this activational effect is not mediated via the Qa-2 molecule.

As a general rule, antibodies against H-2 are not directly mitogenic for T cells. However, analysis of a panel of mAb in our assays demonstrated a two- to threefold proliferative response to many mAb. However, this effect was by far the most pronounced when using the UC3-10H3 mAb, which generally induced responses above two- to fivefold. Activation can also be demonstrated by production of lymphokine after stimulation with the mAb. Additional analysis with both TCR-$\alpha\beta$ and TCR-$\gamma\delta$ T cell clones revealed both were stimulated to proliferate and produce lymphokines in the absence of other cell types, indicating that different types of T cells had the potential to respond to this mAb.

The physiologic significance of these observations remains unclear. It remains possible that signal transduction is not occurring directly through the class I molecule itself, but through a neighboring molecule on the cell surface. However, the mounting evidence that anti-class I mAb can deliver a signal to T cells (12, 13) suggests a potential regulatory role of class I on T cells. The production of IFN-γ or other lymphokines may be a localized response to this stimulus, which would have the effect of up-regulating the expression of class I on the presenting cells nearby, and providing a more efficient immunologic response.

Alternatively, binding of class I mAb may mimic interaction of class I molecules on stimulator cells with TCR. Anti-class II mAb have been shown to deliver signals to B cells (29, 30). Our demonstration of anti-class I-mediated activation of T cells may reflect a more universal functional role of class I in signal transduction. Additional experiments will focus on the activation of non-T cells by anti-class I mAb.

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