Analysis of T Cell Signaling by Class I MHC Molecules: The Cytoplasmic Domain Is Not Required for Signal Transduction

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Summary

The structural requirements for signal transduction by class I major histocompatibility complex (MHC) molecules were examined. Native or mutant HLA-A2 or HLA-B27 constructs lacking most of their cytoplasmic domains were co-transfected with pSV2neo into Jurkat cells. Transfection of either native or mutant constructs resulted in a comparable expression of the gene products. Stimulation of transfectants expressing either native or truncated A2 or B27 molecules with specific mAb evoked an increase in $\left[\text{Ca}^{2+}\right]_i$ upon crosslinking. Moreover, crosslinking native or truncated A2 or B27 induced IL-2 production upon co-stimulation with phorbol myristate acetate. These results confirm that crosslinking class I MHC molecules transduces an activation signal to human T cells. Effective signaling was observed when all but four of the intracytoplasmic residues were deleted, indicating that signal transduction does not require this portion of the molecule.

The role of class I MHC molecules in restricting T cell-mediated cytotoxicity is well established (1). Recently, a role for class I MHC molecules as signal-transducing structures on human T cells has also been demonstrated (2, 3). Thus, crosslinking mAb to monomorphic or polymorphic determinants of class I MHC molecules expressed by highly purified human peripheral blood T cells, Jurkat T cell leukemia cells, or human T cell clones resulted in an increase in intracellular calcium ($\left[\text{Ca}^{2+}\right]_i$) and IL-2 production with appropriate co-stimulation. Since certain functions of class I MHC molecules have been shown to require the integrity of the cytoplasmic domain (4), the present study was carried out to study the role of the cytoplasmic domain of class I MHC molecules in signal transduction and activation of T cells. Wild-type or mutant HLA-A2 or HLA-B27 genes were transfected into Jurkat cells, and the capacity of the transfected cells to be activated upon cross-linking with mAb to polymorphic determinants encoded by the transfected genes was examined. Effective T cell signaling was observed when all but four of the intracytoplasmic residues were deleted, indicating that signal transduction does not require this portion of the molecule.

Materials and Methods

mAbs. A variety of mAbs were used, including: 64.1, directed at the CD3 molecular complex; W6/32 and MB40.5, directed at monomorphic MHC class I-encoded gene products; TM-1 (a gift from Dr. Elisabeth Weiss, Institute for Immunology, University of Munich, Munich, West Germany), directed at an HLA-B27 determinant (5); MA2.1, directed at HLA-A2 or B17 (6); and PA2.1, directed at an HLA-A2 determinant (7). IgG2a mAb P1.17 and IgG1 mAb MOPC-31C were used as controls.

DNA Constructs. Site-directed mutagenesis was carried out as previously described (8) on genomic clones encoding HLA-A*0201 (the gift of Dr. H.T. Orr, University of Minnesota, Minneapolis, MN) or HLA-B*2705. Mutagenesis of HLA-A2 was carried out on a 1.8-kb BglII-SphI fragment containing exons 4–7, subcloned into M13mp19. Mutagenesis of HLA-B27 was carried out on a 2.3-kb AvrII-PstI fragment containing exons 4–8, subcloned into M13mp19. A2 and B27 exon 5 stop codon mutants (A21-312, B271-312) with a Ser→Asn substitution at position 312 and Ser→stop codon at position 313 were constructed. Successful mutagenesis was confirmed by oligonucleotide hybridization followed by DNA sequencing. The double-stranded mutated inserts were then isolated and ligated into pUC9 (HLA-A2) or pUC19 (HLA-B27) constructs that contained the remainder of the respective genes. The presence of the correct mutations was confirmed by DNA sequencing of the recombinant plasmids in both orientations.

DNA Transfection. DNA-mediated gene transfer into Jurkat cells was accomplished by electroporation. Genes of interest were co-transfected with pSV2neo and cells were selected for neo gene expression with G418. In some experiments transfected cells were cloned before use.

Cell Culture Techniques. The cells were incubated with saturating concentrations of mAbs for 30 min at 4°C, washed, and incubated in microtiter wells (No. 3596; Costar, Cambridge, MA), with or without affinity-purified goat anti-mouse Ig (GaMlg, 1 μg/ml) and PMA (1 ng/ml) for 24 h at 37°C, after which the culture su-
pernats were harvested and tested for the presence of IL-2 by the use of CTLL cells, as described (3). All data are calculated from the mean cpm of triplicate determinations and are expressed as U/ml of IL-2 as compared with a standard curve.

**Analysis of Cytosolic Free Calcium ([Ca\(^{2+}\)]).** Flow cytometry was performed as described (2) to analyze the change in [Ca\(^{2+}\)]\(_i\) of the cell population after loading the cells with the calcium sensitive dye, indo-1.

**Results**

**Expression of Native or Mutant HLA-A2 or HLA-B27 Genes by Transfected Jurkat Cells.** Control Jurkat cells expressed neither HLA-A2 or HLA-B27 (Fig. 1). Transfection with either native or truncated genes resulted in expression of A2 or B27 molecules. Immunoprecipitation of surface iodinated molecules and analysis by PAGE confirmed that the transfectants expressed class I MHC heavy chains of the predicted size and that the expressed heavy chains were associated with \(\beta_2\)-microglobulin (data not shown).

**Crosslinking Native or Mutant Class I MHC Molecules Induces a Rise in [Ca\(^{2+}\)]\(_i\).** Crosslinking the native or the mutant class I MHC molecules induced an increase in [Ca\(^{2+}\)]\(_i\). As shown in Fig. 2, >60% of Jurkat cells expressing either A2 or truncated A2\(_{1-312}\) generated a rapid and sustained increase in [Ca\(^{2+}\)]\(_i\) upon crosslinking with GaM1g. Similar results were observed with B27 and B27\(_{1-312}\) (data not shown). By contrast, no change in [Ca\(^{2+}\)]\(_i\) was induced in control Jurkat cells.

**Crosslinking Native or Truncated Class I MHC Molecules Induces IL-2 Production.** Jurkat cells produce IL-2 when CD3 is engaged and the cells are co-stimulated with PMA. Similarly, crosslinking class I MHC molecules and co-stimulating with PMA induces IL-2 production. Crosslinking native or truncated A2 induced IL-2 production upon co-stimulation with PMA (Table 1). Comparable results were observed when truncated B27 molecules were crosslinked with specific mAbs (Table 1). In contrast, neither MA2.1 nor TM-1 induced IL-2 production by control Jurkat (data not shown).

**Table 1. Crosslinking Native or Truncated HLA-A2 or B27 Molecules Induces IL-2 Production by Transfected Jurkat Cells**

| Transfected gene | mAb specificity | IL-2 production | U/ml |
|------------------|-----------------|-----------------|------|
|                  |                 |                 |      |
| A2               | Control         | 0.6             | 0.6  |
|                  | CD3             | 6.1             | 2.7  |
|                  | HLA-A,B,C       | 0.5             | 5.4  |
|                  | HLA-A2          | 0.5             | 3.8  |
|                  | CD3             | 1.5             | 1.6  |
|                  | A2\(_{1-312}\)  | 0.6             | 15.0 |
|                  | CD3             | >16.0           | 6.6  |
|                  | HLA-A2          | 0.5             | 0.5  |
|                  | CD3             | >10.0           | >10.0|
|                  | HLA-A,B,C       | 0.5             | >10.0|
|                  | HLA-B27         | 0.8             | 9.2  |
|                  | CD3             | >10.0           | >10.0|
|                  | HLA-A,B,C       | 0.5             | >10.0|
|                  | HLA-B27         | 0.8             | 9.2  |

**Discussion**

The experiments described in this communication were undertaken to determine the structural requirements for signal transduction by class I MHC molecules. The results indicate that the cytoplasmic portion of class I MHC molecules is not required for signal transduction.

Transfection of Jurkat cells with either the wild-type or the truncated A2 or B27 constructs resulted in stable cell surface expression of the appropriate molecules. This observation indicates that no more than four amino acids of the cy-
The cytoplasmic domain is required for transport to and stable expression of class I MHC molecules on the cell surface, and is in accordance with previous observations of normal expression of truncated H-2Ld molecules transfected into murine L cells (9).

Normal expression of a variety of other transmembrane molecules including CD2, CD4, CD3-e and I-A has also been noted when various portions of the cytoplasmic domains were deleted (10-13). Thus, the cytoplasmic domains of a variety of functionally important transmembrane molecules expressed on lymphoid cells are not required for expression. The cytoplasmic portion of the molecule may be important for other aspects of their function, however. One aspect of the function of transmembrane molecules that frequently appears to require the intracytoplasmic domain is the capacity to transmit activation signals to lymphocytes. This conclusion is supported by the observations that truncated CD2 and CD4 do not function as effective signal generating molecules (10, 11, 14). The signaling capacity of class I MHC molecules differed from that of CD2 or CD4 in that the cytoplasmic domain did not appear to be required. These results imply that signal transduction by class I MHC molecules differs from that induced by engaging CD2 or CD4. Moreover, the results indicate that signal transduction has different structural requirements than other functional activities of class I MHC molecules. For example, constitutive endocytosis of class I MHC molecules via coated pits requires residues 323-340 (exon 7) of the cytoplasmic domain (4). The current results, therefore, not only imply that different functional activities manifest different structural requirements, but also that constitutive endocytosis is not required for class I MHC molecules to transduce activation signals.

The cytoplasmic domain of class I MHC undergoes phosphorylation, the significance of which is unclear. The major in vivo site is serine 335, which is conserved between human and murine molecules (15). In addition, there is a highly conserved single tyrosine residue in position 320 of the cytoplasmic domain (16). The results of the current studies indicate that neither serine nor tyrosine phosphorylation of the cytoplasmic tail is involved in T cell activation induced by cross-linking class I MHC molecules.

The current findings do not establish the mechanism by which class I MHC molecules transduce activation signals. One possibility is that crosslinking class I MHC molecules induces association with other cell surface proteins that transmit activation signals. Since the ability of truncated H-2Ld molecules to undergo capping is comparable to native molecules (9), they are likely to retain the capacity to interact with the cytoskeleton. Such an association might play a role in establishing a macromolecular complex, including class I MHC molecules, involved in signal transduction. In conclusion, the results confirm that crosslinking class I MHC molecules induces activation of T cells and co-stimulates IL-2 production. Deletion of the cytoplasmic portion of the molecule, which includes highly conserved and seemingly important domains and phosphorylation sites, did not affect the ability of the mutant constructs to deliver an activation signal. Class I MHC molecules, therefore, differ from CD2 and CD4 in which the cytoplasmic domain is required for signal transduction.

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