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Cutting Edge: MHC Class I Triggering by a Novel Cell Surface Ligand Costimulates Proliferation of Activated Human T Cells[1]

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BY55 is a human cell surface molecule whose expression is restricted to NK cells, a subset of circulating CD8$^+$ T lymphocytes, and all intestinal intraepithelial T lymphocytes. Here, we report that BY55 is a novel NK receptor showing broad specificity for both classical and nonclassical MHC class I molecules, and that optimal binding requires a prior aggregation of MHC class I complexes. Using BY55 transfectants, we have identified functional consequences of MHC class I/ligand interactions for the class I-bearing cell. The triggering of MHC class I molecules on human T cell clones by BY55 delivered a potent proliferative signal in the presence of soluble CD3 mAb. The costimulatory signal provided by MHC class I ligation was only seen in activated, and not resting, peripheral blood T cells. This observation represents an additional and/or alternative pathway to CD28 costimulation and may be of particular relevance in memory T cells lacking CD28, such as intestinal intraepithelial T lymphocytes, which are CD28$^-$ but BY55$^+$. *The Journal of Immunology*, 1999, 162: 1223–1226.

We previously described an IgM mAb that allowed the identification of an 80-kDa human cell surface structure termed BY55 (1). The BY55 molecule is expressed on CD56^{dim+} NK cells, most TCR$\gamma\delta$-expressing lymphocytes (1), a subset of CD8$^{bright+}$ circulating T cells (2), and all intestinal intraepithelial T lymphocytes (3). The BY55 molecule identifies all lymphocytes with cytotoxic activity in PBLs and human umbilical cord blood (4). We recently cloned BY55 cDNA, and the sequence predicts a cysteine-rich, glycosylphosphatidylinositol-anchored molecule with a single Ig-like domain. DNA database searches did not identify any related human sequences, whereas protein searches revealed weak homology to the first Ig-C2 domain of the killer inhibitory receptor (KIR),[3] KIR2DL4: 22% identity and 44% similarity (3). KIRs deliver inhibitory signals to the lytic machinery of NK cells after binding to specific MHC class I molecules on target cells (5, 6). To date, no role for BY55 in NK and CTL cytotoxicity has been identified (1, 4), which is in keeping with its lack of immunoreceptor tyrosine-based inhibitory and activating motifs (7). We speculated that if BY55 was also an MHC class I ligand, it might lead to signaling in the MHC class I-bearing "target" cell. The ligation of T cell MHC class I molecules by mAbs in vitro can lead to inhibition (8, 9), proliferation (10, 11) or anergy/apoptosis (12, 13).

Here, we show that the BY55 molecule is a ligand for classical and nonclassical MHC class I. Most importantly, ligation of T cell MHC class I complexes by BY55 triggers a proliferative second signal to human T cell clones in the presence of soluble anti-CD3 mAb, whereas CD28 costimulation is ineffective.

Materials and Methods

Cells

The human T cell clones TC5 (CD4$^+$CD8$^+$CD28$^-$) (14), NAR-1 (CD4$^+$) and C-16 (CD8$^+$) (15), and Cou LS (a human T cell lymphoma cell line) (14) were described previously. JAF43 is a CD4$^+$ alloreactive T cell clone. T cells were purified by negative selection from normal PBLs using magnetic bead sorting (Miltenyi Biotec, Bergisch Gladbach, Germany). Chinese hamster ovary (CHO) cells were transfected with BY55 (CHO-BY55) (3), CD86 (CHO-CD86), or vector (CHO-mock) (16). J26 are murine L cells expressing human β_2-microglobulin (β_2m), whereas J26-B7 and J26-HLA-G also express human HLA-B7 and HLA-G, respectively (17). J26-HLA-E are J26 cells transfected with HLA-E containing cd3.14 cosmid (kindly donated by M. Ulbrecht, Institut für Anthropologie and Human Genetik, Munich, Germany).

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[3] Abbreviations used in this paper: KIR, killer inhibitory receptor; CHO, Chinese hamster ovary; β_2m, β_2-microglobulin.

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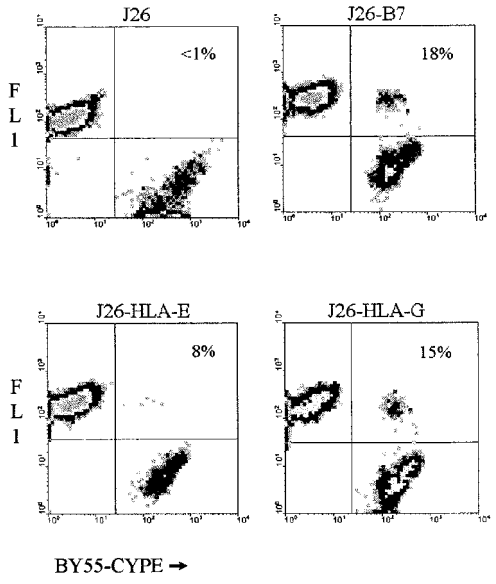

FIGURE 1. CHO-BY55 transfectants form conjugates with murine cells expressing classical or nonclassical human MHC class I molecules. CHO-BY55 and partner cells were centrifuged together and incubated at 37°C for 20 min. Murine transfectants were stained with anti-H-2K^k mAb followed by FITC-labeled goat anti-mouse Ig. BY55-CYPE was added after indirect staining. Ab labeling was performed after conjugate formation to avoid possible effects of mAbs on conjugate formation. The results expressed in the upper right quadrant are the percentage of CHO-BY55-forming conjugates. CHO-CD86 did not form conjugates with either J26-B7 or J26-HLA-G (data not shown).

Abs and tetramers

We purchased CD3-Tricolor (Caltag, Burlingame, CA), CD45-FITC (Immunotech, Marseille, France), and BY55-CYPE (Coulter, Miami, FL). Other mAbs were produced locally or obtained through exchanges of the Fifth International Workshop on the Differentiation Ags (18). Specifically, the IgM anti-BY55 mAb and CLI-R2, an IgG1 mAb, are directed against different epitopes on the BY55 molecule.

Recombinant human HLA-A2 tetramers were produced as described previously (19) and cross-linked with anti-HLA-A2 mAb (BB7.2).

Conjugate formation

A total of 2×10^5 CHO-BY55 and partner cells were centrifuged together and incubated at 37°C for 20 min. Subsequently, anti-BY55-CYPE and an FITC-conjugated mAb (specific for the partner cell) were added for 15 min, and the cells were washed and analyzed for double-stained cells.

Proliferation assays

A total of 10^2–10^4 irradiated (100 Gy) CHO-BY55, CHO-CD86, or CHO-mock cells were plated in 96-well round-bottom plates. The T cell clones were used 7 days after stimulation with feeder cells. Purified PBL T cells were used immediately after sorting. A total of 5×10^4 cells per well were cultured in triplicate with: medium alone, soluble CD3 mAb (1.7 μg/ml of CD3 $\times$ 3, produced in our laboratory), IL-2 (50 U/ml, Eurocetus, Amsterdam, The Netherlands), or 1 μg/ml PHA-M (Sigma, St. Louis, MO). [^{3}H]TdR incorporation was measured as described previously (14).

Results and Discussion

BY55 transfectants form conjugates with murine cells expressing classical and nonclassical human MHC class I molecules

Because of the homology of BY55 with KIRs, we examined whether BY55 also bound to MHC class I. We performed flow cytometry to detect conjugate formation between CHO-BY55 and murine L cells expressing human β_2m and HLA-B7, HLA-E, or HLA-G. CHO-BY55 bound to HLA-B7, HLA-E, and HLA-G transfectants but not to L cells expressing β_2m alone (Fig. 1). The relatively low level of conjugate formation is attributable to the weak expression of human MHC class I by the transfectants.

Optimal conjugate formation requires prior aggregation of MHC class I molecules

We found that CHO-BY55 formed conjugates with human cell lines, regardless of their MHC class I haplotype (Fig. 2*a* shows representative examples). The specificity of the binding was confirmed by inhibition with CLI-R2 mAb, which is an IgG1 mAb directed against BY55 but not an isotype-matched control (L161, anti-CD1c) (data not shown). Interestingly, experiments designed to block CHO-BY55 binding with the monomorphic anti-MHC class I mAb W6/32 led to increased conjugate formation (Fig. 2*b*). This effect was specific, as anti-CD2 mAbs of various isotypes failed to increase conjugate formation between CHO-BY55 and the CD2$^+$ Cou LS cell line (data not shown). The MHC class I$^-$ Daudi cell line did not form conjugates with CHO-BY55 in the presence or absence of W6/32 mAb (Fig. 2, *a* and *b*). These results suggested that BY55 molecules bound to classical and nonclassical MHC class I, and that optimal binding required a cross-linking of MHC class I complexes.

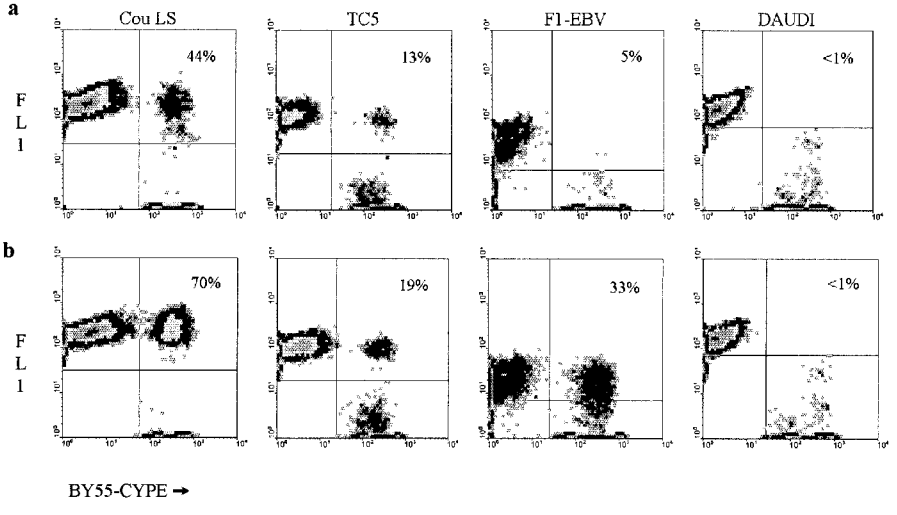

FIGURE 2. Cross-linking of partner cell MHC class I molecules augments binding of BY55. Conjugate formation occurred as described in the legend to Fig. 1. The percentage of CHO-BY55-forming conjugates is indicated in the upper right quadrant. Staining was performed with BY55-CYPE and CD45-FITC (read in the FL1 channel). *a*, Cou LS (an MHC class II$^-$ T cell line), TC5 (a CD4$^+$CD8$^+$ T cell clone), FI-EBV (an EBV-transformed lymphoblastoid cell line), and Daudi (an MHC class I$^-$ cell line). *b*, Partner cells were incubated with the MHC class I mAb, W6/32, for 10 min, washed, and mixed with CHO-BY55.

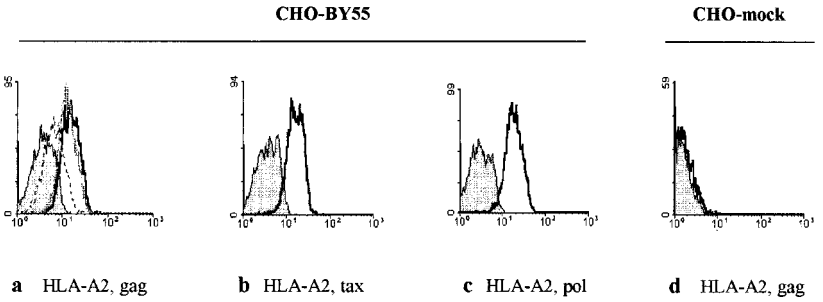

FIGURE 3. Cross-linked HLA-A2 tetramers specifically bind to BY55. CHO-BY55 were stained with tetramers that had been cross-linked with anti-HLA-A2 mAb. *a*, HLA-A2 folded with the HIV peptide, gag (solid black line), and the specificity was tested by preincubating CHO-BY55 with either IgM anti-BY55 mAb (solid gray line) or CLI-R2, an IgG1 anti-BY55 mAb (dotted line). *b*, HLA-A2 with the human T lymphotropic virus type-1 peptide, tax. *c*, HLA-A2 with the HIV peptide, pol. *d*, CHO-mock cells were stained with HLA-A2, gag. Controls (HLA-A2 tetramers alone) are represented by the shaded curves.

Soluble human HLA molecules specifically bind to BY55

To definitively establish that BY55 binds to MHC class I molecules, we used soluble, recombinant, phycoerythrin-labeled human HLA-A2 tetramers refolded with different peptides. HLA-A2 tetramers alone were unable to stain CHO-BY55 as detected by flow cytometry. However, the cross-linking of tetramers with anti-HLA-A2 mAb led to a strong staining of CHO-BY55, regardless of the bound peptide (Fig. 3). The specificity of the binding was shown by inhibition with CLI-R2 mAb, whereas the IgM anti-BY55 was ineffective (Fig. 3*a*); furthermore, CHO-BY55 was unable to bind aggregated Ig (FITC-conjugated anti-HLA-A2 mAb cross-linked with 7S goat anti-mouse Ig). CHO-CD86 or CHO-mock cells were not stained by the tetramers, even when cross-linked (Fig. 3*d*).

Thus, BY55 is a unique NK/TCR with broad specificity for classical and nonclassical MHC class I (similar to the Ig-like transcript-4 expressed on human myelomonocytic cells) (20). Whereas CD94/NKG2A, B, and C NK cell receptors bind soluble HLA-E tetramers but not monomers (21), BY55 requires a further degree of ligand aggregation, namely cross-linking of HLA-A2 tetramers (Fig. 3). Similarly, optimal conjugate formation between CHO-BY55 and other cells required an aggregation of MHC class I (Fig. 2*b*).

Ligation of T cell MHC class I molecules by BY55 delivers a potent costimulatory signal to activated human T cells

As the BY55 molecule is glycosylphosphatidylinositol-linked (3) and does not have intracytoplasmic immunoreceptor tyrosine-based inhibitory and activating motifs, this may explain why no role for BY55 in

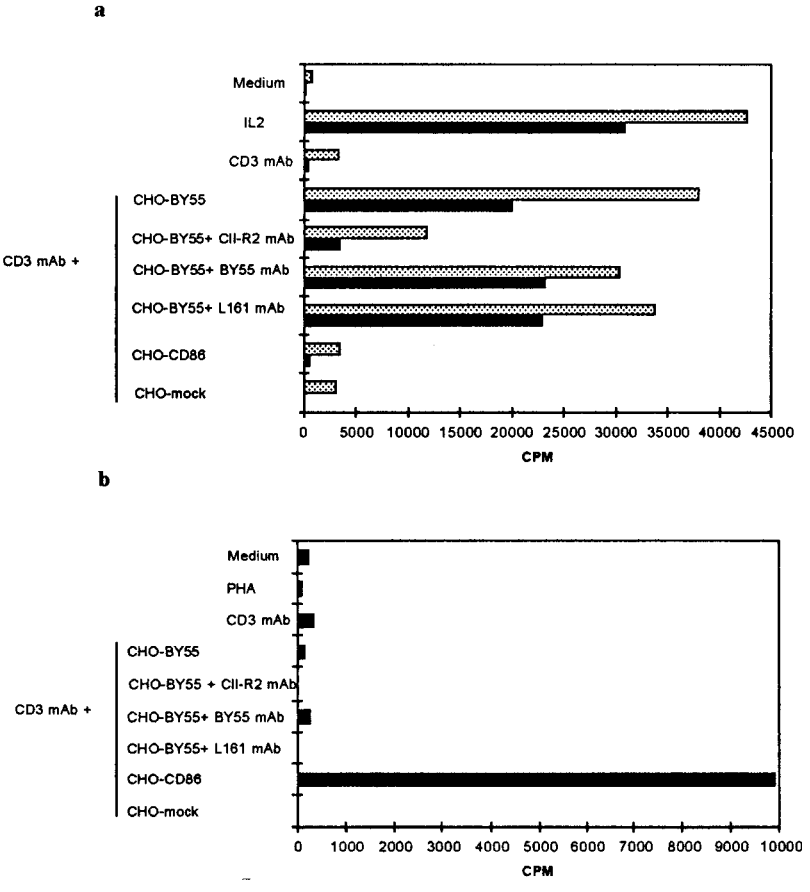

FIGURE 4. Triggering of MHC class I complexes by BY55 costimulates proliferation of activated T cells. A total of 5×10^4 T cells were stimulated as described in *Materials and Methods*. CLI-R2 (an IgG1 anti-BY55 mAb), IgM anti-BY55 mAb, and an IgG1 control (L161, anti-CD1c) were added at the start of cultures (1 in 10,000 dilution of ascites). The background cpm of irradiated CHO cells cultured alone have been subtracted. Results are representative of four separate experiments. *a*, T cell clones: TC5 (black bars) and NAR-1 (gray bars). Similar results were obtained with C-16 and JAF43. *b*, Peripheral blood T cells (>96% $CD3^+$).

NK cytotoxicity has been observed (1, 4). We hypothesized that the BY55/MHC class I interaction might deliver signals to the MHC class I-bearing target cell. We performed proliferation assays with human T cell clones of various phenotypes to examine the effect of triggering their MHC class I complexes with CHO-BY55. Soluble anti-CD3 mAb alone did not induce proliferation of the clones (Fig. 4*a*). When added to soluble anti-CD3, CHO-BY55 but not CHO-CD86 or CHO-mock triggered a strong proliferation of T cell clones. This effect was specifically inhibited by CLI-R2 and not by the IgM anti-BY55 mAb or an isotype control (Fig. 4*a*). CHO-BY55 alone did not induce proliferation. CHO-CD86 and soluble anti-CD3 did not induce proliferation in the T cell clones, as they were $CD28^-$ or expressed low levels of CD28. In contrast, T cells from normal PBLs did not proliferate in response to soluble anti-CD3 and CHO-BY55; however, as expected, these T cells did respond to the classical combination of CD3 activation and CD28 costimulation (22) (by soluble anti-CD3 and CHO-CD86) (Fig. 4*b*). Thus, ligation of MHC class I delivers a costimulatory signal specifically to activated T cells, as quiescent peripheral blood T lymphocytes did not respond to a combined triggering of TCR/CD3 and MHC class I complexes. Confirming these observations, we found that when peripheral blood T cells were driven to proliferate by anti-CD3 mAb coated onto culture wells, the addition of CHO-BY55 led to a significant amplification of the proliferative response (data not shown).

Further work is necessary to identify which MHC class I heavy chain domain is involved in the liaison with the BY55 molecule and which signal transduction pathways are involved. The monomorphic MHC class I binding receptor CD8 binds to the $\alpha 3$ domain of MHC class I molecules (23), and mAbs specific for the $\alpha 3$ domain can inhibit T cell activation (24) and induce apoptosis (13). In contrast, cross-linking MHC class I complexes with CHO cells expressing human CD8 was found to augment the IL-2 secretion of activated $CD4^+$ T cells (25). Abs against the HLA class I $\alpha 1$ domain preferentially induced apoptosis in activated T cells (26). MHC class I ligation by mAbs leads to tyrosine kinase phosphorylation, which appears to involve the transduction components of the TCR/CD3 complex (27).

The physiological function of MHC class I triggering by BY55 molecules is unknown, but it may have a particular role in intestinal intraepithelial T lymphocytes that have an activated/memory phenotype such as: $CD8^+$, $CD45RO^+$, $CD45RA^-$, $CD28^-$, $BY55^+$, and MHC class I^+ (3). What compensates for the loss of CD28, the major costimulatory molecule, has been perplexing. Our findings provide a molecular basis for lymphocyte-lymphocyte interactions between BY55 and MHC class I molecules, which could be responsible for an efficient immune response in effector cells in the absence of CD28 costimulation and may play a role in the maintenance of memory.

In conclusion, activated/memory lymphocytes, such as T cell clones that express low levels of CD28, can be driven to proliferate by a second signal from their MHC class I molecules cross-linked by a natural ligand, BY55. Thus, in addition to Ag presentation, MHC class I molecules are also directly involved in T cell costimulation.

References

1. Maiza, H., G. Leca, I. G. Mansur, V. Schiavon, L. Boumsell, and A. Bensussan. 1993. A novel 80-kDa cell surface structure identifies human circulating lymphocytes with natural killer activity. *J. Exp. Med. 178:1121.*
2. Bensussan, A., C. Rabian, V. Schiavon, D. Bengoufa, G. Leca, and L. Boumsell. 1993. Significant enlargement of a specific subset of $CD3^+CD8^+$ peripheral blood leukocytes mediating cytotoxic T-lymphocyte activity during human immunodeficiency virus infection. *Proc. Natl. Acad. Sci. USA 90:9427.*
3. Anumanthan, A., A. Bensussan, L. Boumsell, A. Christ, R. Blumberg, S. Voss, M. Robertson, L. Nadler, and G. Freeman. 1998. Cloning of BY55, a killer inhibitory receptor-related protein expressed on NK cells, cytolytic T lymphocytes, and intestinal intraepithelial lymphocytes. *J. Immunol. 161:2780.*
4. Bensussan, A., E. Gluckman, S. el Marsafy, V. Schiavon, I. G. Mansur, J. Dausset, L. Boumsell, and E. Carosella. 1994. BY55 monoclonal antibody delineates within human cord blood and bone marrow lymphocytes distinct cell subsets mediating cytotoxic activity. *Proc. Natl. Acad. Sci. USA 91:9136.*
5. Lanier, L. L. 1997. Natural killer cells: from no receptors to too many. *Immunity 6:371.*
6. Moretta, A., R. Biassoni, C. Bottino, D. Pende, M. Vitale, A. Poggi, M. C. Mingari, and L. Moretta. 1997. Major histocompatibility complex class I-specific receptors on human natural killer and T lymphocytes. *Immunol. Rev. 155:105.*
7. Renard, V., A. Cambiaggi, F. Vely, M. Blery, L. Olcese, S. Olivero, M. Bouchet, and E. Vivier. 1997. Transduction of cytotoxic signals in natural killer cells: a general model of fine tuning between activatory and inhibitory pathways in lymphocytes. *Immunol. Rev. 155:205.*
8. Amirayan, N., C. Vernet, and P. Machy. 1992. Class I-specific antibodies inhibit proliferation in primary but not secondary mouse T cell responses. *J. Immunol. 148:1971.*
9. Amirayan, N., and P. Machy. 1996. MHC class I molecules are implicated in costimulatory signals during TCR/CD3-induced activation. *Cell. Immunol. 168:1.*
10. Roosnek, E., A. Tunnacliffe, and A. Lanzavecchia. 1990. T cell activation by a bispecific anti-CD3/anti-major histocompatibility complex class I antibody. *Eur. J. Immunol. 20:1393.*
11. Bushkin, Y., S. Demaria, N. Mohagheghpour, and J. M. Le. 1990. Activation of human $CD8^+$ T cells via the CD8/HLA class I complex. *Cell. Immunol. 126:185.*
12. Skov, S., P. Klausen, and M. H. Claesson. 1997. Ligation of major histocompatability complex (MHC) class I molecules on human T cells induces cell death through PI-3 kinase-induced c-Jun NH2-terminal kinase activity: a novel apoptotic pathway distinct from Fas-induced apoptosis. *J. Cell. Biol. 139:1523.*
13. Sambhara, S. R., and R. G. Miller. 1991. Programmed cell death of T cells signaled by the T cell receptor and the $\alpha 3$ domain of class I MHC. *Science 252:1424.*
14. Bagot, M., H. Echchakir, F. Mami-Chouaib, M.-H. Delfau-Larue, D. Charue, A. Bernheim, S. Chouaib, L. Boumsell, and A. Bensussan. 1998. Isolation of tumor-specific cytotoxic $CD4^+$ and $CD4^+CD8^{dim+}$ T cell clones infiltrating a cutaneous T cell lymphoma. *Blood 91:4331.*
15. Minty, A., S. Asselin, A. Bensussan, D. Shire, N. Vita, A. Vyakarnam, J. Wijdenes, P. Ferrara, and D. Caput. 1997. The related cytokines interleukin-13 and interleukin-4 are distinguished by differential production and differential effects on T lymphocytes. *Eur. Cytokine Netw. 8:203.*
16. Gimmi, C. D., G. J. Freeman, J. G. Gribben, G. Gray, and L. M. Nadler. 1993. Human T-cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. *Proc. Natl. Acad. Sci. USA 90:6586.*
17. Bensussan, A., I. G. Mansur, V. Mallet, A. M. Rodriguez, M. Girr, E. H. Weiss, G. Brem, L. Boumsell, E. Gluckman, J. Dausset, et al. 1995. Detection of membrane-bound HLA-G translated products with a specific monoclonal antibody. *Proc. Natl. Acad. Sci. USA 92:10292.*
18. Boumsell, L. 1995. T-cell antigens: section report. In *Leukocyte Typing V.* S. Schlossman, L. Boumsell, W. Gilks, J. Harlan, T. Kishimoto, C. Morimoto, J. Ritz, S. Shaw, R. Silverstein, T. Springer, T. Tedder, and R. Todd, eds. Oxford University Press, Oxford.
19. Altman, J. D., P. A. H. Moss, P. J. R. Goulder, D. H. Barouch, M. G. McHeyzer-Williams, J. I. Bell, A. J. McMichael, and M. M. Davis. 1996. Phenotypic analysis of antigen-specific T lymphocytes. *Science 274:94.*
20. Colonna, M., J. Samaridis, M. Cella, L. Angman, R. L. Allen, C. A. O'Callaghan, R. Dunbar, G. S. Ogg, V. Cerundolo, and A. Rolink. 1998. Human myelomonocytic cells express an inhibitory receptor for classical and nonclassical MHC class I molecules. *J. Immunol. 160:3096.*
21. Braud, V. M., D. S. Allan, C. A. O'Callaghan, K. Soderstrom, A. D'Andrea, G. S. Ogg, S. Lazetic, N. T. Young, J. I. Bell, J. H. Phillips, L. L. Lanier, and A. J. McMichael. 1998. HLA-E binds to natural killer cell receptors CD94/NKG2A, B, and C. *Nature 391:795.*
22. Gimmi, C. D., G. J. Freeman, J. G. Gribben, K. Sugita, A. S. Freedman, C. Morimoto, and L. M. Nadler. 1991. B-cell surface antigen B7 provides a costimulatory signal that induces T cells to proliferate and secrete interleukin 2. *Proc. Natl. Acad. Sci. USA 88:6575.*
23. Zamoyska, R. 1994. The CD8 coreceptor revisited: one chain good, two chains better. *Immunity 1:243.*
24. Smith, D. M., J. A. Bluestone, D. R. Jeyarajah, M. H. Newberg, V. H. Engelhard, J. R. Thistlethwaite, Jr., and E. S. Woodle. 1994. Inhibition of T cell activation by a monoclonal antibody reactive against the $\alpha 3$ domain of human MHC class I molecules. *J. Immunol. 153:1054.*
25. Geppert, T. D., H. Nguyen, and P. E. Lipsky. 1992. Engagement of class I major histocompatibility complex molecules by cell surface CD8 delivers an activation signal. *Eur. J. Immunol. 22:1379.*
26. Genestier, L., R. Paillot, N. Bonnefoy-Berard, H. Waldmann, and J. P. Revillard. 1997. T cell sensitivity to HLA class I-mediated apoptosis is dependent on interleukin-2 and interleukin-4. *Eur. J. Immunol. 27:495.*
27. Skov, S., S. Bregenholt, and M. H. Claesson. 1997. MHC class I ligation of human T cells activates the ZAP70 and $p56^{lck}$ tyrosine kinases, leads to an alternative phenotype of the TCR/CD3 ζ-chain, and induces apoptosis. *J. Immunol. 158:3189.*