Soluble HLA Class I Molecules/CD8 Ligation Trigger Apoptosis of CD8⁺ Cells by Fas/Fas-Ligand Interaction

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Received November 29, 2001; Accepted January 4, 2002; Published February 12, 2002

KEY WORDS: soluble HLA, apoptosis, Fas ligand, CD8

DOMAINS: inflammation, cell cycle (fate), hematology

The human major histocompatibility complex (HLA) encodes two sets of HLA class I molecules, which have been termed class Ia (or classical) and class Ib (or nonclassical) molecules. The class Ia molecules include the gene products of HLA-A, HLA-B, and HLA-C loci and are characterized by broad tissue expression and by a high degree of polymorphism. The class Ib molecules include the gene products of HLA-E, HLA-F, and HLA-G loci and are characterized by a restricted tissue distribution and by limited polymorphism. Besides being expressed on nucleated cells, classical and nonclassical HLA class I molecules are present in serum in soluble form (sHLA-I)[1,2]. The serum level of sHLA-I molecules is significantly increased in a variety of physiological and pathological conditions such as pregnancy, acute rejection episodes following organ allografts, acute graft-versus-host-disease (GVHD) following bone marrow transplantation, autoimmune diseases, viral infections, and malignant melanoma[3,4,5,6]. Because of the statistically significant association with clinical parameters, the level of sHLA-I antigens has been suggested to represent a useful marker to predict the evolution of viral infections and to monitor the clinical course of allografts[7]. Moreover, elevated levels of functional sHLA-I and soluble Fas-ligand molecules have been detected by our group in blood components and might play a role in the immunomodulatory effect of autologous and allogeneic transfusions[8,9,10,11]. Several lines of evidence suggest that sHLA-I molecules are immunologically functional and may play an immunoregulatory role. In fact, they have been shown to elicit antibodies in both allogeneic and xenogeneic combinations, to inhibit the activity of alloreactive cytotoxic T lymphocytes (CTL)[12,13,14], and to induce apoptosis in alloreactive and virus-specific CTL, in activated autologous and allogeneic CD8⁺ T cells, and in CD8⁺ NK cells[15,16,17,18,19]. There is general agreement about the mechanism underlying the inhibition of CTL activity by sHLA antigens. This inhibition appears to be mediated by interactions of sHLA-I antigens α_1 and α_2 domains with T cell receptor (TCR)[12,13,14]. By contrast, there is conflicting information about the mechanism underlying induction of apoptosis of activated T cells by sHLA-I antigens. Several authors reported that sHLA-I molecules induced apoptosis of alloreactive CD8⁺ cytotoxic T lymphocytes through interaction with their TCR[15]. However, our own data and those other groups indicate that classical and nonclassical sHLA-I molecules trigger

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Fas/Fas-ligand mediated apoptosis of phytohemagglutinin (PHA)-activated and virus-specific CD8⁺ T lymphocytes as well as of CD8⁺ NK cells by interacting with CD8 coreceptor [17,18,19,20]. Recently, we performed a series of experiments in our laboratory to clarify the intracellular mechanism(s) leading to Fas-ligand upregulation and secretion. These unpublished data indicate that sHLA-I/CD8 ligation elicits the phosphorylation of p56^{lck} protein tyrosin kinase (PTK) associated with CD8 cytoplasmic domain in the absence of any other TCR-derived signal, the activation of *syk*-like ZAP-70 PTK and protein kinase C, and extracellular calcium influx. Then, activation and nuclear translocation of NF-κB and NF-AT occurs, leading to Fas-ligand mRNA transcription and soluble Fas-ligand secretion, which delivers the death signal. Interestingly, soluble Fas-ligand secretion and CD8⁺ cell apoptosis, but not CD8⁺ cell cytolytic activity, are completely inhibited by Cyclosporin A, which specifically blocks the activation of the calcineurin/calmodulin pathway. Taken together, these data suggest that sHLA-I molecules are involved in a signal-transduction pathway leading to Fas-ligand expression, soluble Fas-ligand secretion, and CD8⁺ cells apoptosis.

Apoptosis induced by Fas/Fas-ligand interactions plays a crucial role for the establishment of antigen-specific T cell tolerance, both during the intrathymic negative selection and during adult life [21,22,23,24,25]. The amount of sHLA-I molecules that induces apoptosis in CD8⁺ cells is analogous to the level found in plasma of patients with an activation of their immune system. Therefore, sHLA-I antigens secreted during immune system activation may bind to CD8 molecules on activated CD8⁺ cells and induce soluble Fas-ligand secretion. Then, soluble Fas-ligand may act in an autocrine and/or paracrine way, triggering apoptosis in activated CD8⁺ CD95⁺ cells. If so, serum sHLA-I molecules may represent an important efferent arm of the network to control the expansion of CD8⁺ cells and to downregulate immune responses and could be proposed as potential immunosuppressive tools in the field of transplantation and autoimmunity.

REFERENCES

1. Van Rood, J.J., van Leeuwen, A., and van Santen, M.C.T. (1970) Anti HL-A2 inhibitor in normal human serum. *Nature* **226**, 366–367.
2. Charlton, R.K. and Zmijewski, C.M. (1970) Soluble HL-A7 antigen: localization in the β-lipoprotein fraction of human serum. *Science* **170**, 636–637.
3. Puppo, F., Brenci, S., Lanza, L., Bosco, O., Imro, M.A., Scudeletti, M., Indiveri, F., and Ferrone, S. (1994) Increased level of serum HLA Class I antigens in HIV infection. Correlation with disease progression. *Hum. Immunol.* **40**, 259–266.
4. Puppo, F., Scudeletti, M., Indiveri, F., and Ferrone, S. (1995) Serum HLA class I antigens: markers and modulators of an immune response? *Immunol. Today* **16**, 124–127.
5. Hunt, J.S., Jadhav, L., Chu, W., Geraghty, D.E., and Ober, C. (2000) Soluble HLA-G circulates in maternal blood during pregnancy. *Am. J. Obstet. Gynecol.* **183**, 682–688.
6. Ugurel, S., Rebmann, V., Ferrone, S., Tilgen, W., Grosse-Wilde, H., and Reinhold, U. (2001) Soluble human leukocyte antigen-G serum level is elevated in melanoma patients and is further increased by interferon-alpha immunotherapy. *Cancer* **92**, 369–376.
7. Puppo, F., Indiveri, F., Scudeletti, M., and Ferrone, S. (1997) Soluble HLA antigens: new roles and uses. *Immunol. Today* **18**, 154–155.
8. Ghio, M., Contini, P., Mazzei, C., Brenci, S., Barberis, G., Filaci, G., Indiveri, F., and Puppo F. (1999) Soluble HLA class I, HLA class II and Fas ligand in blood components: a possible key to explain the immunomodulatory effects of allogeneic blood transfusions. *Blood* **93**, 1770–1777.
9. Ghio, M., Contini, P., Mazzei, C., Brenci, S., Filaci, G., Indiveri, F., and Puppo, F. (2000) Soluble HLA class I and Fas ligand molecules in blood components and their role in the immunomodulatory effects of blood transfusions. *Leuk. Lymphoma* **37**, 1–8.
10. Puppo, F., Ghio, M., Contini, P., Mazzei, C., and Indiveri F. (2001) Fas, Fas ligand, and transfusion immunomodulation. *Transfusion* **41**, 416–418.
11. Ghio, M., Contini, P., Mazzei, C., Merlo, A., Filaci, G., Setti, M., Indiveri, F., and Puppo, F. (2001) In vitro immunosuppressive activity of soluble HLA class I and Fas ligand molecules: do they play a role in autologous blood transfusion? *Transfusion* **41**, 988–996.

12. Hausmann, R., Zavazava, N., Steinmann, J., and Müller-Ruchholtz, W. (1993) Interaction of papain-digested HLA class I molecules with human alloreactive cytotoxic T lymphocytes (CTL). *Clin. Exp. Immunol.* **91**, 183–188.
13. Parham, P., Clayberger, C., Zorn, S.L., Ludwig, D.S., Schoolnik, G.K., and Krensky, A.M. (1987) Inhibition of alloreactive cytotoxic T lymphocytes by peptides from the α_2 domain of HLA-A2. *Nature* **325**, 625–628.
14. Dal Porto, J., Johansen, T.E., Catipovic, B., Parfiit, D.J., Tuveson, D., Gether, U., Kozlowski, S., Fearon, D.T., and Schneck, J.P. (1993) A soluble divalent class I major histocompatibility complex molecule inhibits alloreactive T cells at nanomolar concentrations. *Proc. Natl. Acad. Sci. U. S. A.* **90**, 6671–6675.
15. Zavazava, N. and Krönke, M. (1996) Soluble HLA class I molecules induce apoptosis in alloreactive cytotoxic T lymphocytes. *Nat. Med.* **2**, 1005–1010.
16. Contini, P., Ghio, M., Merlo, A., Brenci, S., Filaci, G., Indiveri, F., and Puppo, F. (2000) Soluble HLA class I/CD8 ligation triggers apoptosis in EBV-specific CD8⁺ cytotoxic T lymphocytes by Fas/Fas-ligand interaction. *Hum. Immunol.* **61**, 1347–1351.
17. Gansuvd, B., Haghiara, M., Munkhbat, B., Kanai, N., Morita, N., Munkhtuvshin, N., Chargui, J., Kato, S., Hotta, T., and Tsuji, K. (2000) Inhibition of Epstein-Barr virus (EBV)-specific CD8⁺ cytotoxic T lymphocyte (CTL) activity by soluble HLA class I in vitro. *Clin. Exp. Immunol.* **119**, 107–114.
18. Puppo, F., Contini, P., Ghio, M., Brenci, S., Scudeletti, M., Filaci, G., Ferrone, S., and Indiveri, F. (2000) Soluble human MHC class I molecules induce soluble Fas ligand secretion and trigger apoptosis in activated CD8⁺ Fas(CD95)⁺ T lymphocytes. *Int. Immunol.* **12**, 195–203.
18. Spaggiari, G.M., Contini, P., Carosio, R., Arvigo, M., Ghio, M., Oddone, D., Dondero, A., Zocchi, M.R., Puppo, F., Indiveri, F., and Poggi, A. (2001) Soluble HLA class I molecules induce Natural Killer cell apoptosis through the engagement of CD8. Evidence for a negative regulation exerted by CD94/NKG2A complex and KIR2D. *Blood*, in press.
20. Fournel, S., Aguerre-Girr, M., Huc, X., Lenfant, F., Alam, A., Toubert, A., Bensussan, A., and Le Bouteiller, P. (2000) Soluble HLA-G1 triggers CD95/CD95 ligand-mediated apoptosis in activated CD8⁺ cells by interaction with CD8. *J. Immunol.* **164**, 6100–6104.
21. Mountz, J.D., Zhou, T., Wu, J., Wang, W., Su, X., and Cheng, J. (1995) Regulation of apoptosis in immune cells. *J. Clin. Immunol.* **15**, 1–16.
22. van Parijs, L. and Abbas, A.K. (1996) Role of Fas-mediated cell death in the regulation of immune responses. *Curr. Opin. Immunol.* **8**, 355–361.
23. Yonehara, S., Nishimura, Y., Kishil, S., Yonehara, M., Takazawa, K., Tamatani, T., and Ishii, A. (1994) Involvement of apoptosis antigen Fas in clonal deletion of human thymocytes. *Int. Immunol.* **6**, 1849–1856.
24. Lynch, D.H., Ramsdell, F., and Alderson, M.R. (1995) Fas and FasL in the homeostatic regulation of immune responses. *Immunol. Today* **16**, 569–574.
25. Kurts, C., Heath, W.R., Kosaka, H., Miller, J.F.A.P., and Carbone, F. (1998) The peripheral deletion of autoreactive CD8⁺ T cells induced by cross-presentation of self-antigens involves signaling through CD95 (Fas, Apo-1). *J. Exp. Med.* **188**, 415–420.

This article should be referenced as follows:

Puppo, F., Contini, P., Ghio, M., and Indiveri, F. (2002) Soluble HLA class I molecules/CD8 ligation trigger apoptosis of CD8⁺ cells by Fas/Fas-ligand interaction. *TheScientificWorldJOURNAL* **2**, 421–423.

Handling Editor:

Charles Serhan, Principal Editor for *Inflammation* — a domain of *TheScientificWorldJOURNAL*.