MHC class II engagement inhibits CD99-induced apoptosis and up-regulation of T cell receptor and MHC molecules in human thymocytes and T cell line

Min Kyung Kim[a,1], Yoon-La Choi[a], Min Kyung Kim[a], Seok-Hyung Kim[a], Eun Young Choi[a], Won Seo Park[b], Young Mee Bae[b], Sang Kyu Woo[c], Seong Hoe Park[a,*]

[a] *Department of Pathology, College of Medicine, Seoul National University, Yongun-dong 28, Chongro-gu, Seoul 110-799, South Korea*
[b] *Department of Pathology, Kangwon National University College of Medicine, 192-1 Hyoja 2-dong, Chunchon, Kangwon-do 200-701, South Korea*
[c] *DiNonA, Inc., 65 Umyeon-dong, Seocho-gu, Seoul 137-140, South Korea*

Received 6 February 2003; revised 23 April 2003; accepted 2 May 2003

First published online 10 June 2003

Edited by Beat Imhof

Abstract Major histocompatibility complex (MHC) class II surface levels on thymocytes increase after CD99 ligation. The functional implication of the up-regulated MHC class II was assessed by engaging MHC class II on CD99-ligated cells. MHC class II engagement down-modulated surface levels of T cell receptor and MHC molecules, and inhibited apoptosis of CD99-ligated thymocytes and CEM tumor cells, antagonistic effects on the previously reported CD99 functions. The results were reproducible regardless of the order of ligation of MHC class II and CD99. We suggest that signaling via MHC class II on CD99-engaged cells might be involved in the thymic maturation process by damping CD99 ligation effects.

Key words: Major histocompatibility complex class II; CD99; T cell receptor; Apoptosis; Thymocyte; Selection

1. Introduction

During the maturation process, most immature thymocytes are presumed to die as a result of lack of positive selection that occurs when T cell receptors (TCRs) recognize peptide/major histocompatibility complex (MHC) complexes expressed on selecting cells, such as thymic epithelial cells and human thymocytes, with appropriate affinity [1–4]. Along the development process in the thymus, the expression level of TCR complex on thymocytes is quantitatively regulated with an increase after positive selection [5].

A 32 kDa surface glycoprotein, CD99, is expressed highly in immature thymocytes and T cells but moderately in many other hematopoietic cell types [6,7]. The antigen has been presumed to be involved in various steps of T cell development and differentiation. While CD99 ligation provides peripheral blood T cells with a co-stimulatory signal in the presence of suboptimal CD3 stimulation and leads to Th1 cell differentiation of the engaged T cells [8,9], it stimulates homotypic aggregation [10] and apoptosis [11] of thymocytes. Moreover, CD99 ligation on immature thymocytes induces up-regulation of MHC molecules and TCR expression on the cells, suggesting that CD99 is a cell surface protein with a potential modulatory effect on positive selection by enhancing the efficiency of TCR–MHC interaction [12].

We previously reported that MHC class II molecules are expressed in significant amounts on human fetal and postnatal thymocytes [13], which is not the case in mice. However, the biological relevance of MHC class II expression on human immature and mature T cells is not well understood. MHC class II^+ double positive (DP) thymocytes are able to positively select DP thymocytes, leading to differentiation into $CD4^+CD8^-$ and $CD4^-CD8^+$ cells in the in vitro reaggregate culture system [4]. In this system, differentiation into single positive (SP) T cells was significantly blocked by incubation with anti-MHC class II monoclonal antibodies (mAbs), suggesting a role of MHC II antigens on thymocytes in positive selection in the human system.

In this paper, to elucidate the functional role of MHC class II expressed on a human tumor T cell line and thymocytes, we investigated phenotypic changes related to CD99 function in immature T cells by incubating cells with anti-HLA-DR mAbs in addition to anti-CD99 mAb. We show that MHC class II might play a role in the thymic maturation process, exerting a negative influence on CD99 functioning.

2. Materials and methods

2.1. Cells

CEM T cells were maintained at 37°C and 5% CO_2 in RPMI 1640 (Gibco, Grand Island, NY, USA), supplemented with 10% fetal calf serum (FCS; Gibco), penicillin at 400 U/ml, and streptomycin at 150 μg/ml. Human thymuses were obtained from donors undergoing cardiac surgery at Seoul National University Hospital and were processed for single cell suspension of thymocytes as previously described [12].

2.2. Antibodies

For engagement, cells were incubated with unconjugated forms of anti-MHC class II (YG18 or 2.06), anti-MHC class I (W6/32), or anti-CD99 (DN16) mAbs and goat anti-mouse IgG for cross-linking. Anti-CD1a mAb (OKT6) was used for an isotype-matched control. To

*Corresponding author. Fax: (82)-2-763 6625.
E-mail address: pshoe@plaza.snu.ac.kr (S.H. Park).

[1] Present address: Department of Pathology, Ilsan Paik Hospital, Inje University College of Medicine, 2240 Daewha-dong, Ilsan-gu, Koyang-si, Kyungki-do, South Korea.

Abbreviations: MHC, major histocompatibility complex; TCR, T cell receptor; PI, propidium iodide; APC, antigen-presenting cell

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00567-2

detect antigens on the cells engaged with unconjugated forms of mAbs described above, fluorescein isothiocyanate (FITC)-conjugated mAbs of different epitopes for MHC class II (L243), MHC class I (YG13), or CD99 (YG32) were used for staining. The unconjugated forms of mAbs (YG18, 2.06, W6/32, DN16, and OKT6) and FITC-conjugated mAbs (YG13, L243, DN16 and YG32) were purchased from DiNonA (Seoul, Korea). Anti-TCR α-FITC mAb was purchased from PharMingen (San Diego, CA, USA). Propidium iodide (PI; Becton Dickinson, Sunnyvale, CA, USA) and FITC-conjugated annexin V (PharMingen) were used at 50 μg/ml and at 1 μg/ml, respectively.

2.3. Cell engagement with monoclonal antibodies

2×10^6 cells from CEM T cells and thymocytes were placed into wells of 24-well tissue culture plates (Corning, Corning, NY, USA) containing 10% fetal bovine serum–RPMI culture medium and incubated for 30 min at 37°C in the presence of anti-CD99 (DN16: 1 μg/ml) mAb, and then incubated with an isotype-matched control (OKT6: 10 μg/ml) or anti-MHC class II (YG18: 10 μg/ml) mAbs for an appropriate time (1–30 h). The primary antibodies were cross-linked by 5–10 μg/ml of goat anti-mouse IgG.

2.4. Flow cytometric analysis

After engagement with appropriate antibodies, CEM T cells or thymocytes were washed with phosphate-buffered saline (PBS) twice and finally with PBS supplemented containing 1% FCS and 0.05% sodium azide (FACS buffer), and were incubated for 30 min on ice with FITC-conjugated mAbs. After washing twice with the FACS buffer, cells were analyzed on a FACSCalibur (Becton Dickinson). For apoptosis analysis, engaged cells were washed twice with cold PBS and resuspended in HEPES buffer (10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$) at a concentration of 1×10^6 cells/ml. Cells were incubated with annexin V–FITC and PI for 15 min at room temperature prior to analysis on FACSCalibur.

3. Results

3.1. MHC class II engagement down-regulates CD99-induced up-regulation of TCR

To investigate the role of MHC class II antigen expressed on human T cells, we treated a CEM human T cell line culture with anti-HLA-DR (YG18) in conjunction with anti-CD99 (DN16) mAb plus secondary Ab and checked whether addition of anti-MHC class II mAb would have any effect on the surface level of TCR on CD99-engaged cells. The phenomenon of up-regulated TCR expression on CD99-engaged cells [12] was recapitulated in CEM cells, in a dose-dependent manner as well (Fig. 1A). However, co-engagement of MHC class II and CD99 on CEM cells abolished this phenomenon, with no effect detected from the cells treated with isotype-matched control Ab (OKT6) plus anti-CD99 mAb (Fig. 1B). It was anti-HLA-DR Ab-specific, since treatment of anti-MHC class I Ab (W6/32) did not show the same phenomenon, while another clone of anti-MHC class II mAb (2.06) also showed blocking effect (Fig. 1C). This antagonizing effect of HLA-DR engagement on CD99-induced TCR up-regulation was observed regardless of the timing of anti-HLA-DR Ab treatment in relation to CD99 engagement (Fig. 1D) and continued up until 30 h after initiation of CD99 engagement (Fig. 1E). This confirms that the blocking effect was not because anti-HLA-DR mAb occupied secondary Ab necessary for CD99 engagement.

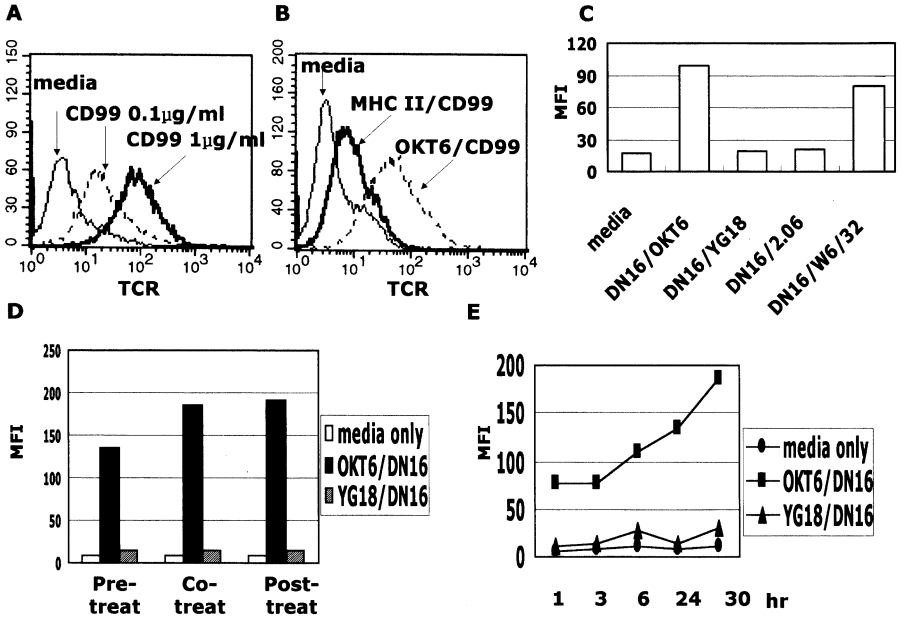

Fig. 1. MHC class II engagement inhibits CD99-induced up-regulation of TCR on CEM T cells. A: CEM T cells were engaged with indicated concentrations of anti-CD99 mAb (DN16) or with medium only for 2 h. Then the cells were stained with FITC-conjugated anti-TCR mAb and analyzed on a FACSCalibur. B: After engagement with anti-MHC II mAb (YG18) or an isotype-matched control mAb (OKT6) for 30 min, cells were incubated with anti-CD99 mAb (DN16) for 1 h and then analyzed for TCR expression. C: After engagement with anti-CD99 mAb (DN16) for 30 min, cells were engaged for 2 h with indicated clones of anti-MHC II mAb (YG18 or 2.06), anti-MHC I mAb (W6/32), or a control Ab (OKT6). The cells were analyzed for TCR surface level using flow cytometry and mean fluorescence intensity (MFI) values are plotted. D: Engagement with anti-MHC class II mAb (YG18) or a control Ab (OKT6) was followed by (pre-treat), simultaneously done with (co-treat), or preceded by (post-treat) engagement with anti-CD99 mAb (DN16). After incubation for 24 h, cells were analyzed for TCR surface level and the MFI values were compared. E: After pre-treatment with anti-MHC II mAb (YG18) or a control Ab (OKT6) for 30 min, cells were engaged with anti-CD99 mAb (DN16) and then analyzed for TCR levels at indicated time points. The concentrations of Abs used in engagement are 10 μg/ml for YG18, 2.06, OKT6 and W6/32, and 1 μg/ml for DN16 all through the experiments, unless otherwise indicated. A–E: representative data of three independent experiments.

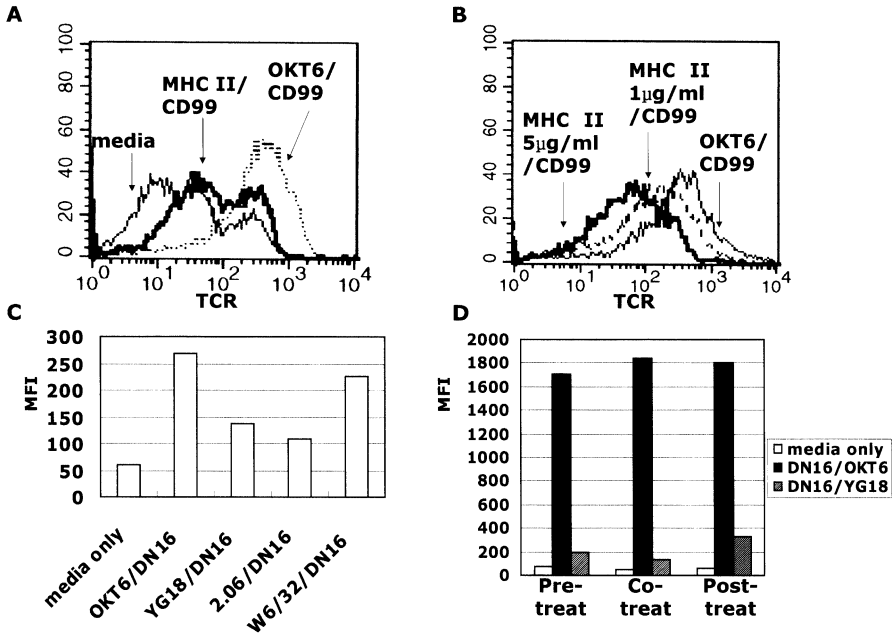

Fig. 2. MHC class II engagement inhibits CD99-induced up-regulation of TCR on human thymocytes. A: Thymocytes were treated as described in Fig. 1B and analyzed for TCR expression using flow cytometry. B: Thymocytes were engaged with indicated concentrations of anti-MHC II mAb (YG18) or a control Ab (OKT6) for 30 min and then anti-CD99 mAb (DN16) for 2 h. C,D: Thymocytes were treated and analyzed for TCR expression as described in Fig. 1C,D. A–D: representative data of three independent experiments.

To see whether the same phenomenon could be observed in thymocytes, a population of which expresses HLA-DR naturally [13], thymocytes were treated in the same way. As seen in CEM cells, pre-incubation with anti-HLA-DR Abs (YG18 or 2.06) blocked up-regulation of TCR induced by CD99 engagement (Fig. 2A), more effectively with an increased amount of the Ab added for pre-incubation (Fig. 2B), whereas treatment of anti-MHC class I Ab did not show the same phenomenon (Fig. 2C). This antagonizing effect of HLA-DR engagement on CD99-induced TCR up-regulation was independent of the order of engagement of MHC class II and CD99 on thymocytes (Fig. 2D).

3.2. *MHC class II ligation inhibits CD99-induced up-regulation of MHC class II and MHC class I molecules*

MHC class I and II molecules are also up-regulated on thymocytes by CD99 engagement [12]. To examine whether the blocking effect of anti-HLA-DR mAb would be relevant to MHC class I and II antigens, surface levels of MHC molecules were checked on CEM cells treated in the same way. To detect MHC antigens on the cells engaged with anti-CD99 (DN16) plus anti-HLA-DR (YG18) or anti-MHC class I (S6/32) mAbs, FITC-conjugated mAbs of different binding specificity for MHC class I (YG13) and MHC class II (L243) were used for flow cytometric analysis. Up-regulation of MHC class II antigen, which was supposed to occur by CD99 engagement, was suppressed by pre-treatment with anti-HLA-DR mAb (YG18: Fig. 3A,B). The inhibitory effect was HLA-DR-specific and more prominent on MHC class II antigen level, compared to that on MHC class I antigen (Fig. 3C,D). This antagonizing effect of HLA-DR engagement on CD99-induced MHC class II up-regulation was reproduced regardless of the timing of anti-HLA-DR Ab treatment in relation to CD99 engagement (Fig. 3E) and persisted up to 30 h (Fig. 3G), whereas the inhibitory effect on MHC class I

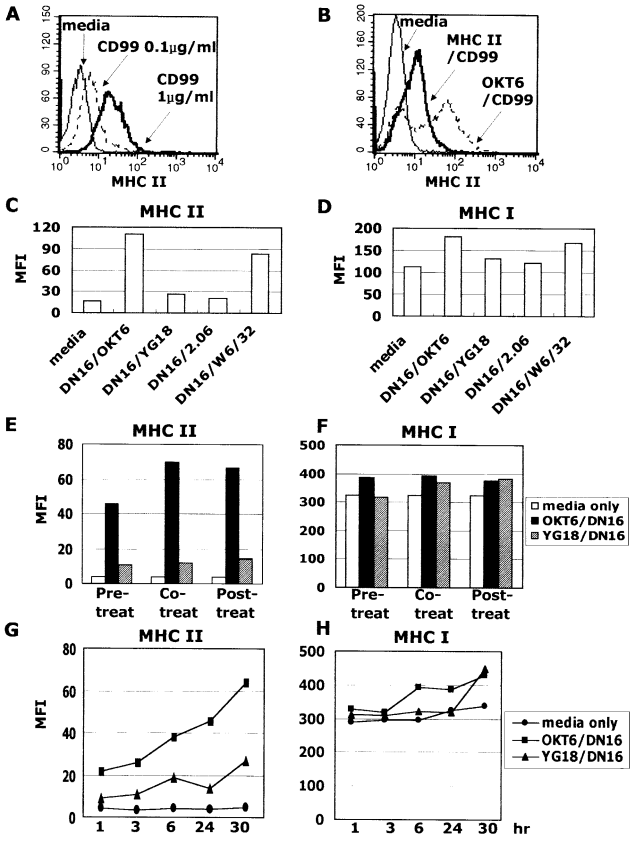

Fig. 3. MHC class II engagement inhibits CD99-induced up-regulation of MHC class II and MHC class I on CEM T cells. CEM T cells were engaged in the same way as described in Fig. 1A–E, and analyzed for MHC class II (A–C,E,G) and for MHC class I (D,F,H) surface expression.

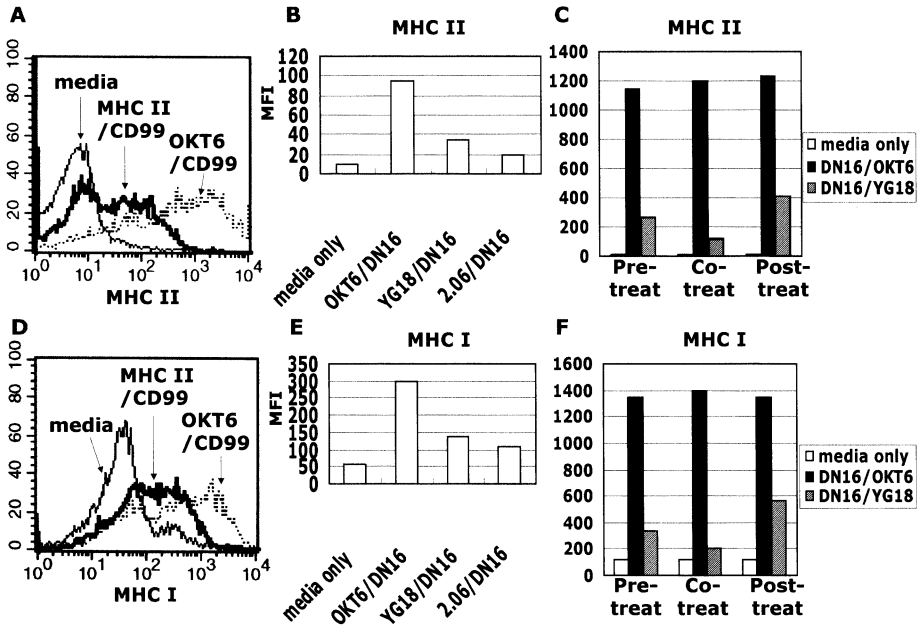

Fig. 4. MHC class II engagement inhibits CD99-induced up-regulation of class II and MHC class I on human thymocytes. Thymocytes were treated in the same way as described in Fig. 2 and analyzed for MHC class II (A–C) and for MHC class I (D–F).

expression was not remarkable (Fig. 3F,H). Based on the fact that CD99-induced up-regulation of surface antigens is mediated via enhanced transport of the antigens present in cytoplasm to cell surface [12], we suggest that the insignificant increase of the surface level of MHC class I after CD99 engagement and unremarkable blocking effect of HLA-DR after co-engagement with CD99 (Fig. 3D, F, and H) might be due to the constitutive expression of the antigen on CEM cells at a high level.

To substantiate the idea, we checked thymocytes, on which surface levels of MHC class I and II are low to moderate, with the same method. Up-regulation of both MHC class I and class II by CD99 engagement was reduced when anti-HLA-DR mAb YG18 (Fig. 4A,D) or 2.06 (Fig. 4B,E) was treated before CD99 engagement, which was independent of the timing of anti-HLA-DR mAb treatment in relation to CD99 engagement (Fig. 4C,F).

This result shows that treatment of thymocytes with anti-

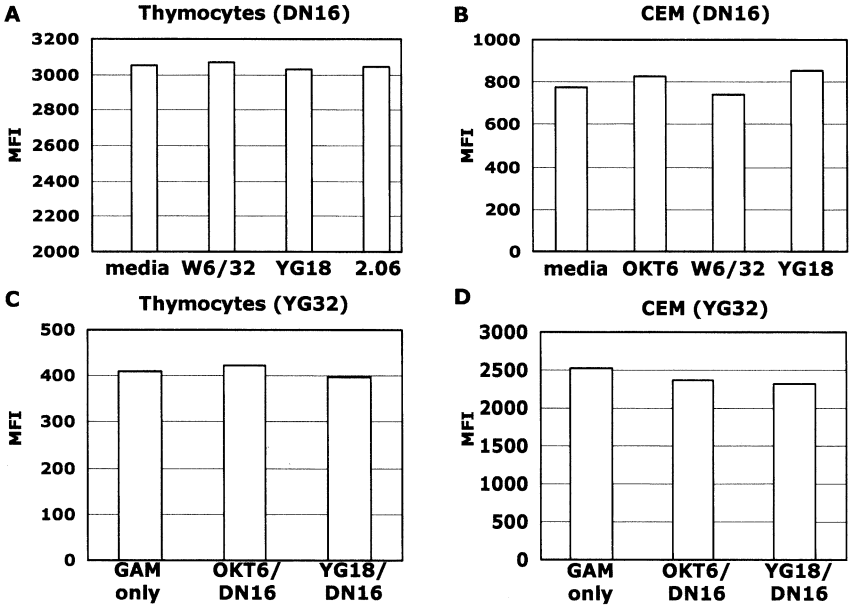

Fig. 5. MHC class II engagement does not affect CD99 expression. Thymocytes (A) and CEM T cells (B) were engaged with or without indicated clones of anti-MHC II mAbs (YG18 or 2.06), anti-MHC I mAb (W6/32) alone for 30 min. Then, the cells were stained with DN16-FITC mAb and analyzed on FACSCalibur. The MFI values were plotted. Thymocytes (C) and CEM T cells (D) were co-engaged with DN16 (anti-CD99 mAb) plus YG18 (anti-MHC II mAb) or OTK6 (a control Ab), or treated with goat anti-mouse IgG (GAM) only for 2 h. Then, the cells were analyzed for CD99 surface level after staining with FITC-conjugated YG32 (anti-CD99) mAb and the MFI values were plotted. Those are representative data of three independent experiments.

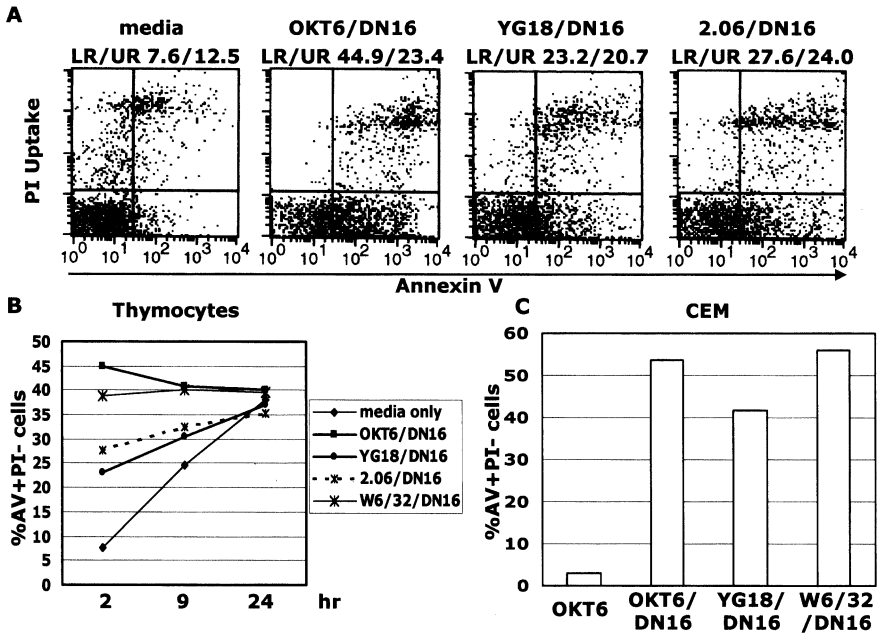

Fig. 6. Inhibition of CD99-induced apoptosis by MHC class II engagement in human thymocytes and CEM T cells. A: Thymocytes engaged with anti-MHC II mAb (YG18 or 2.06) or a control Ab (OKT6) for 30 min and then with anti-CD99 mAb (DN16) for 2 h were incubated for 15 min with annexin V–FITC and PI and analyzed on a FACSCalibur. The numbers at the top of the dot plots indicate the percentage of cells in the lower right (LR)/upper right (UR) quadrants, respectively. B: Time courses of apoptosis in thymocytes treated as above were plotted. C: The same assay was performed on CEM T cells and then early apoptotic cell populations (annexin V^+ PI^-) were compared. The data are representative of three independent experiments.

HLA-DR mAb (YG18 or 2.06) inhibits CD99-induced up-regulation of all three antigens involved in T–antigen-presenting cell (APC) interaction, TCR and MHC class I and II antigens.

3.3. MHC II ligation does not affect CD99 expression

One possible explanation for the inhibitory effect of anti-HLA-DR Ab could be that MHC class II engagement induced down-regulation of the surface level of CD99 antigen. To test this, CEM T cells and human thymocytes incubated with appropriate mAbs were analyzed for CD99 surface level by flow cytometry. Cells engaged with anti-HLA-DR mAb (YG18 or 2.06) did not show any change in CD99 surface level as compared to that after engaging cells with control mAbs (OKT6 and W6/32) (Fig. 5A,B). Co-engagement of the cells with anti-HLA-DR mAb YG18 and anti-CD99 mAb DN16 did not affect the surface level of CD99 antigen either (Fig. 5C,D), as detected at a similar level to that after control co-engagement (YG18 plus control Ab OKT6) by staining with FITC-conjugated anti-CD99 mAb YG32, of which the binding specificity is different from that of DN16 [14]. This result shows that the inhibition of CD99-induced up-regulation of surface antigen by anti-HLA-DR mAb treatment was a direct effect of the binding of the Ab to the MHC class II antigen, not an indirect one through down-modulation of CD99 antigen.

3.4. Inhibition of CD99-induced apoptosis by MHC class II ligation

Engagement of CD99 induces apoptosis of Jurkat T cells [15], thymocytes [11], and Ewing's sarcoma cells [16]. To test whether prior incubation with anti-HLA-DR mAb has an antagonizing effect against CD99-induced cell apoptosis, thymocytes treated with anti-HLA-DR mAb and anti-CD99 mAb subsequently were checked for apoptosis by flow cytometric analysis using annexin V–FITC and PI to distinguish early and intermediate apoptotic cells (annexin V^+ PI^-) from late phase apoptotic cells and dead cells (annexin V^+ PI^+). The percentage of pro-apoptotic cells (annexin V^+ PI^-) was increased by incubation with anti-CD99 mAb plus control Ab for 2 h compared to untreated thymocytes. However, the percentage was reduced by almost 50% with treatment of anti-HLA-DR mAb in addition to anti-CD99 mAb, which was observed from the cells incubated with another type of anti-HLA-DR mAb (2.06: Fig. 6A). The blocking effect on CD99-induced apoptosis was more obvious at an early incubation time in thymocytes than in CEM cells (Fig. 6B,C). The combined results demonstrate that treatment with anti-HLA-DR mAb plays a significant role in neutralizing or suppressing CD99-induced apoptosis of thymocytes.

4. Discussion

In this paper, we focused on the effect of incubation of anti-HLA-DR mAb on the CD99 ligation-induced phenomenon in a human T tumor cell line and thymocytes. Anti-CD99 mAb engagement induced up-regulation of surface expression of TCR and MHC antigens and apoptosis of thymocytes, however, pre-, co-, or post-incubation of anti-HLA DR mAb abrogated all these CD99-induced phenotypes. This was not because the anti-HLA-DR mAb treatment affected surface expression of CD99, nor did it use up secondary Ab necessary for CD99 ligation. This is anti-HLA-DR Ab-specific, since pre-incubation with anti-MHC class I Ab did not show any significant difference from CD99 engagement alone.

Enhanced intracellular transport to the cell surface is con-

sidered to be a main mechanism for the CD99-induced increase of surface expression of TCR and MHC antigens [13], as supported by the facts of tight association of the TCR complex with the cytoplasmic microtubule network [17] and Rac1 activity-dependent MHC class I and MHC class II up-regulation after CD99 engagement involving microtubule assembly [18–20]. These intracellular events might be associated with signals transduced by CD99 ligation that elevate intracellular Ca^{2+} and enhance TCR-dependent cellular activation [8,9,21]. MHC class II engagement triggers activation events including a cascade of protein tyrosine phosphorylation, phosphatidylinositol turnover, and an increase in cytosolic Ca^{2+} in human B and T cells [22,23]. Surface MHC class II was co-immunoprecipitated with phospholipase Cγl on activated human T cells and the Jurkat T cell line, although the exact mechanism how this complex could be formed is unclear [24]. The lack of precise information as to what kinases are physiologically linked to MHC class II for signal transduction limited our understanding of the linkage between MHC class II and the CD99-induced signaling pathways. Our postulation is that the signals delivered by MHC class II engagement might converge on the microtubule network with those transduced by CD99 engagement, thereby blocking the CD99-induced accelerated transport of TCR and MHC molecules.

A biological role of MHC antigens expressed on APCs is presenting antigenic peptides to T cells, serving as a ligand for the TCR from $CD4^+$ T cells [25]. However, the role of the antigen expressed on T cells during T cell development and activation is not defined well. The MHC class II molecules expressed on activated T cells can transduce signals in T cells to facilitate activation and proliferation in the presence of a suboptimal range of TCR/CD3 stimulation [26,27]. The only result, that treatment of anti-HLA-DR mAb in in vitro thymic reaggregate culture delayed the differentiation of immature thymocytes [4], has suggested the possible importance of MHC class II engagement during thymocyte development and differentiation processes in thymus. The result obtained in this study, inhibition of CD99-induced apoptosis of thymocytes by incubation with anti-HLA-DR mAb, supports the idea of involvement of MHC class II engagement during thymic development. Anti-apoptotic signaling through MHC class II molecules reported in B cell lines and splenic B cells [28] implies that MHC class II signaling is related to anti-apoptosis no matter which cells MHC class II molecules are expressed on. Based on this, biological relevance of the antagonistic effect of MHC class II engagement on CD99 ligation-related phenotypes is proposed that interaction between CD99 and its ligand initiates the up-regulation of surface molecules, thereby enhancing positive selection of immature thymocytes, and induces rapid apoptosis of the cells that are still neglected, but interaction of MHC class II on thymocytes with its ligand, possibly CD4 (according to epitopes of YG18 and 2.06 mAbs), might antagonize the CD99 effect on surface antigens and prolong the life span of immature thymocytes, controlling the overall pace of thymocyte development in thymus. Therefore, we suggest that the whole process of positive selection and maturation of immature thymocytes might be a result of fine-tuning between CD99 signaling and counteracting MHC class II signaling, both of which act to enhance the efficiency of positive selection all together.

Acknowledgements: The study was supported by funds from the 2002 National Research Laboratory Program (M1010400012401J00000551) of the Republic of Korea.

References

[1] Surh, C.D. and Sprent, J. (1994) Nature 372, 100–103.
[2] Kappler, J.W., Roehm, N. and Marrack, P. (1987) Cell 49, 273–280.
[3] Fowlkes, B.J. and Schweighoffer, E. (1995) Curr. Opin. Immunol. 7, 188–195.
[4] Choi, E.Y., Park, W.S., Jung, K.C., Chung, D.H., Bae, Y.M., Kim, T.J., Song, H.G., Kim, S.H., Ham, D.I., Hahn, J.H., Kim, J., Kim, K., Hwang, T.S. and Park, S.H. (1997) Hum. Immunol. 54, 15–20.
[5] Kearse, K.P., Roberts, J.L., Munitz, T.I., Wiest, D.L., Nakayama, T. and Singer, A. (1994) EMBO J. 13, 4504–4514.
[6] Ellis, N.A., Ye, Y.Z., Patton, S., German, J., Goodfellow, P.N. and Weller, P. (1994) Nat. Genet. 6, 394–400.
[7] Bernard, A. (1997) in: Leukocyte Typing VI. Proceedings of the VIth International Leukocyte Differentiation Antigen Workshop, Kobe, Japan, 1996 (Kishimoto, T., Ed.), pp. 75–77, Garland, London.
[8] Waclavicek, M., Majdic, O., Stulnig, T., Berger, M., Sunder Plassmann, R., Zlabinge, G.J., Baumruker, T., Stokl, J., Ebner, C., Knapp, W. and Pickl, W.F. (1998) J. Immunol. 161, 4671–4678.
[9] Wingett, D., Forcier, K. and Nielson, C.P. (1999) Cell. Immunol. 193, 17–23.
[10] Bernard, G., Zoccola, D., Decker, M., Breittmayer, J.P., Aussel, C. and Bernard, A. (1995) J. Immunol. 154, 26–32.
[11] Bernard, G., Breittmayer, J.P., de Matteis, M., Trampont, P., Hofman, P., Senik, A. and Bernard, A. (1997) J. Immunol. 158, 2543–2550.
[12] Choi, E.Y., Park, W.S., Jung, K.C., Kim, S.H., Kim, Y.Y., Lee, W.J. and Park, S.H. (1998) J. Immunol. 161, 749–754.
[13] Park, S.H., Bae, Y.M., Kim, T.J., Ha, I.S., Kim, S., Chi, J.G. and Lee, S.K. (1992) Hum. Immunol. 33, 294–298.
[14] Gil, M.C., Lee, M.H., Seo, J.I., Choi, Y.L., Kim, M.K., Jung, K.C., Park, S.H. and Kim, T.J. (2002) Exp. Mol. Med. 34 (6), 411–418.
[15] Pettersen, R.D., Bernard, G., Olafsen, M.K., Pourtein, M. and Lie, S.O. (2001) J. Immunol. 166, 4931–4942.
[16] Sohn, H.W., Choi, E.Y., Kim, S.H., Lee, I., Chung, D.H., Sung, U.A., Hwang, D.H., Cho, S.S., Jun, B.H., Jang, J.J., Chi, J.G. and Park, S.H. (1998) Am. J. Pathol. 153, 1937–1945.
[17] Tse, D.B., Al-Haideri, M., Pernis, B., Cantor, C.R. and Wang, C.Y. (1986) Science 234, 748–751.
[18] Wubbolts, R., Fernandez-Borja, M., Jordens, I., Reits, E., Dusseljee, S., Echeverri, C., Vallee, R.B. and Neefjes, J. (1999) J. Cell Sci. 112, 785–795.
[19] Sohn, H.W., Shin, Y.K., Lee, I., Bae, Y.M., Suh, Y.H., Kim, M.K., Kim, T.J., Jung, K.C., Park, W.S., Park, C.S., Chung, D.H., Ahn, K., Kim, I.S., Ko, Y.H., Bang, Y.J., Kim, C.W. and Park, S.H. (2001) J. Immunol. 166, 787–794.
[20] Wubbolts, R., Fernandez-Borja, M., Oomen, L., Wrwoerd, D., Janssen, H., Calafat, J., Tulp, A., Dusseljee, S. and Neefjes, J. (1996) J. Cell Biol. 135, 611.
[21] Hahn, M.J., Yoon, S.S., Sohn, H.W., Song, H.G., Park, S.H. and Kim, T.J. (2000) FEBS Lett. 470, 350–354.
[22] Lane, P.J.L., McConnell, F.M., Schieven, G.L., Clark, E.A. and Ledbetter, J.A. (1990) J. Immunol. 144, 3684–3692.
[23] Odum, N., Martin, P.J., Schieven, G.L., Hansen, J.A. and Ledbetter, J.A. (1991) Eur. J. Immunol. 212, 123–129.
[24] King, R.L. and Nguyen, Q.V. (1998) J. Cell Biochem. 70, 346–353.
[25] Germain, R.N. (1994) Cell 76, 287–299.
[26] Holling, T.M., van der Stoep, N., Awinten, E. and van den Elsen, P.J. (2002) J. Immunol. 168, 763–770.
[27] Frosch, S., Bonifas, U., Ross, R., Schwing, J., Yagita, H., Guo, Y., Liu, Y. and Reske-Kunz, A.B. (1996) Immunology 89, 384–390.
[28] Catlett, I.M., Xie, P., Hostager, B.S. and Bishop, G.A. (2001) J. Immunol. 166, 6019–6024.