A Novel Role of Metalloproteinase in Cancer-mediated Immunosuppression

Bor-Ching Sheu, Su-Ming Hsu, Hong-Nerng Ho, Huang-Chun Lien, Su-Cheng Huang, and Rong-Hwa Lin

Departments of Obstetrics and Gynecology, [B-C.S., H-N.H., S-C.H.], Pathology [H-C.L., S-M.H.,] and Graduate Institute of Immunology [H-N.H., S-M.H., R-H.L.], National Taiwan University College of Medicine, Taipei, Taiwan, 100

ABSTRACT

Depressed immune responses have been observed frequently in cancer patients. In a variety of human malignancies, the expression of interleukin-2 receptor α (IL-2Rα) on activated tumor-infiltrating lymphocytes was down-regulated. Because IL-2Rα plays a pivotal role in the development and propagation of functional T cells, its depressed expression may result in poor function of tumor-reactive cytotoxic lymphocytes. For elucidating the mechanism responsible for down-regulation of IL-2Rα, a coculture model of in vitro mixed autologous lymphocytes and tumor cells was established. Kinetic analysis showed that cervical cancer cells down-regulated IL-2Rα expression on encountered T cells. The amount of IL-2Rα mRNA in tumor-infiltrating lymphocytes-derived CD8+ T cells was compatible with that in the corresponding activated CD8+ T cells. Additional evidence showed that cervical cancer cells could induce the release of soluble IL-2Rα expression on encountered T cells. By using protease inhibition assays we demonstrated that tissue inhibitors of metalloproteinase abrogated the cancer-mediated IL-2Rα proteolytic process and restored the T-cell proliferation function. Immunohistochemical stainings further revealed prominent metalloproteinase (MMP) expressions, including MMP-1, MMP-2, and MMP-9, in cervical cancer tissues. Additional in vitro studies showed that MMP-9 mediates cleavage of IL-2Rα and down-regulates the proliferative capability of cancer-encountered T cells. Our findings suggest a new role of MMPs in tumor-mediated immunosuppression and provide a possible therapeutic potential for patients with cervical cancer.

INTRODUCTION

Escape from immune surveillance prefigures the rapid progression of human cancers (1–3). Various immune escape mechanisms in cancer have been proposed (4, 5). Certain cancer cells may secrete immunosuppressive factors to modify the host immune responses (1–5). The impaired antitumor immunity can also be related to defective immune regulation (4). In a variety of human malignancies, the expression of IL-2Rα on activated TILs was down-regulated (6–9). Because IL-2Rα plays a pivotal role in the development and propagation of functional T cells, its depressed expression may result in poor function of tumor-reactive cytotoxic lymphocytes.

Depressed immune responses have been observed in selected patients with various types of tumors, including CC (10–12). Not infrequently, cancer spread occurs despite the apparent presence of TILs (12–14). In human CC as well as in other cancers, tumor-specific autologous cytotoxic T cells are easily identified, but the cytotoxic potential of freshly isolated TILs is usually not expressed (10, 13, 14). This suggests the possible existence of as yet unspecified and perhaps complex immunosuppressive factors or cytokines that affect the anitumor capacity of these TILs in the tumor microenvironment. Recently, we and others have demonstrated that the expression of CD25 (IL-2Rα) on TILs derived from various human cancers was down-regulated (6–9). Because IL-2Rα is an essential receptor for the proliferation of T cells, its depressed expression in vivo may result in poor clonogenicity of cytotoxic lymphocytes (15–18) and cause immune suppression. Possible explanations for down-regulation of IL-2Rα on TILs are as follows: (a) intrinsic defects of IL-2Rα biosynthesis in the T cells of patients with cancer; (b) inhibition of IL-2Rα synthesis in TILs by exogenous mediators derived from cancer cells (7, 9); and/or (c) rapid cleavage of IL-2Rα by intrinsic or extrinsic means within the tumor milieu.

In this study, we used a MLTC model to evaluate the kinetic expression of IL-2Rα on in vitro-activated T cells and to infer the mechanism responsible for down-regulation of IL-2Rα on TILs in human CC. A coculture of MLNC from the same patient was used as control. TILs, cancer cells, and noncancerous cells were isolated by a mechanical dispersal technique as described previously (8, 11). PBMCs from the same patient were used as the source of autologous lymphocytes. We demonstrated for the first time that tumor-derived metalloproteinases can induce the proteolytic cleavage of IL-2Rα on activated T cells and suppress the proliferative capability of cancer-encountered T cells.

MATERIALS AND METHODS

Isolation of TILs, Cancer Cells, and Noncancerous Epithelial and Stromal Cells. Tissue specimens were aseptically excised immediately after surgery from at least four different tumor sites and two sites of normal cervix. Tissue specimens were cut, minced, and pressed gently through a 380-μm sieve and then a 45.7-μm sieve with RPMI 1640 (Life Technologies, Inc., Grand Island, NY), as described previously (8, 11). PBMCs were partially activated with 2 μM of PHA in a 37°C, 5% CO2-humidified incubator for 2 h. The activation and coculture procedures outlined were performed in duplicate in 12 mm × 75 mm capped polystyrene test tubes. In the experimental group (MLTC), 5 × 10^5 activated T cells were cultured with 1 × 10^6 autologous cancer cells (1:2) in 4 ml of culture medium. In the control group (MLNC), 5 × 10^5 activated T cells were cultured with 1 × 10^6 autologous normal cervical stromal/epithelial cells. The immunophenotyping of activated T cells in both groups was analyzed by flow cytometry on days 1, 3, and 5 of coculture. The supernatant was collected at the same intervals and checked for the presence of sIL-2Rα by a standard ELISA (Endogen, Inc., Woburn, MA).

Flow Cytometry Analysis. Monoclonal antibodies labeled with FITC, PE, and PerCP (Becton Dickinson Immunocytochemistry System; Becton Dickinson, Inc., San Jose, CA) were used for three-color flow cytometry. The following matchings were used: (a) anti-CD45-FITC + anti-CD14-PE (leucogate); (b) anti-CD3-FITC + anti-CD19-PE; (c) anti-CD3-FITC + anti-CD4-PE; (d) anti-CD3-FITC + anti-CD8-PE; (e) anti-CD4-FITC + anti-CD25-PE + anti-CD3-PerCP; (f) anti-CD4-FITC + anti-CD25-PE + anti-CD3-PerCP; (g) anti-CD4-FITC + anti-HLA-DR-PE + anti-CD3-PerCP; and (h) anti-CD8-FITC + anti-HLA-DR-PE + anti-CD3-PerCP. A Simulset control (mouse IgGl-FITC + IgG2a-PE) was used as background control. Three-color flow cytometry
was performed on a FACScalibur (Becton Dickinson, Inc.). Leukocyte was used to measure the proportion of lymphocytes in the sample being studied without any scatter gates.

**Purification of CD8+ CTLs.** An indirect magnetic labeling system with a MACS (Miltenyi Biotec, Gladbach, Germany) was used for purification of CD8+ T cells. In brief, the cell suspension was incubated in a cold room (6°C to 12°C) for 10 min with 20 µl of Hepten Antibody Cocktail added (containing CD4, CD11b, CD16, CD19, CD36, and CD56 antibodies; Miltenyi Biotec). After repeated washing, 20 µl of MACS anti-Hepten microbeads/10^7 total cells were added. The magnetically labeled cells were passed through a MACS separator in the magnetic field. The effluent was collected as a negative fraction representing the enriched CD8+ T-cell fraction. The cell purity was checked by flow cytometry after labeling with anti-CD8-PE or FITC. The cells were incubated for an additional 6 h. The activated cells were labeled with monoclonal antibodies for CD4 and CD8. Preparative RT-PCR Analysis. Total cellular RNA of MACS-purified CD8+ T cells was extracted and reverse-transcribed (0.1–0.5 mg of RNA) in the presence of IL-2Rα oligonucleotide primers (Clontech Laboratories, Inc., Palo Alto, CA). First-strand cDNA was synthesized in a DNA thermal cycler (GeneAmp DNA thermal cycler 480; Perkin-Elmer Corp., Norwalk, CT), and the resulting cDNA was amplified by PCR. For competitive analysis of IL-2Rα, another set of synthetic competitors (IL-2Rα mimic primers) with a different length for amplification was used (Clontech Laboratories Inc.). Human β-actin primers and β-actin mimic primers were used as positive controls.

**Preparation of Protease Inhibitors.** Protease inhibitors (Boehringer Mannheim Biochemicals GmbH, Mannheim, Germany, and Calbiochem, La Jolla, CA) for a broad spectrum of inhibition were prepared. The selected protease inhibitors included antipain-dihydrochloride (papain, trypsin, cathepsin A and B-inhibitor), bestatin (aminopeptidase inhibitor), chymostatin (β-, γ-, and δ-chymotrypsin-inhibitor), E-64 (cysteine protease inhibitor), leupeptin (serine and cysteine protease, plasmin, trypsin, papain, and cathepsin B-inhibitor), pepstatin (aspartate protease), aprotinin (serine protease inhibitor, specific for plasmin, kallikrein, trypsin, and chymotrypsin), TIMP-2 (M, 72,000 and M, 92,000 gelatinases inhibitor), and MMP-inhibitor 1 (a broad spectrum gelatinase inhibitor). All reagents were prepared in azide-free deionized H2O. Tritrated concentrations of individual protease inhibitors were prepared according to the recommended working formula of the reagent supplier (Boehringer Mannheim Biochemicals). Drug toxicity was tested by the addition of titrated concentrations of reagents in lymphocyte culture for 24 h with >90% viable cells by the trypan-blue staining method.

**Protease Inhibition Assay.** Different protease inhibitors were added to the established MLTC. For maximal expression of IL-2Rα on the surface of T cells, PBMCs were fully activated with 10 ng/ml PHA for 24 h before MLTC. Autologous cancer cells (1 × 10^6) were first cultured in 1 ml of culture medium containing protease inhibitors. The final concentrations of protease inhibitors as recommended in each tube were as follows: (a) antipain-dihydrochloride, 5 and 50 µg/ml; (b) bapstin, 1 and 10 ng/ml; (c) bestatin, 4 and 40 µg/ml; (d) chymostatin, 6 and 60 µg/ml; (e) E-64, 1 and 10 µg/ml; (f) leupeptin, 1 and 10 µg/ml; (g) pepstatin, 1 and 10 µg/ml; (h) TIMP-2, 0.1 and 1 µg/ml; and (i) MMP-inhibitor 1, 10 and 100 µg/ml. All results were expressed as mean ± SE unless stated otherwise. Data analysis was performed with SAS (Statistical Analysis System, R.6.12; SAS Institute, Inc., Cary, NC). Kinetic data were analyzed by a generalized estimation equation for correlated data of repeated measurements. Statistical significance was defined by a P of <0.05.

**RESULTS AND DISCUSSION**

**CC Cells Can Decrease IL-2Rα Expression on Encountered T Cells.** In the first experiment, PHA-activated PBMCs were cocultured with autologous CC cells or noncancerous cells. The expression of IL-2Rα and HLA-DR on activated T cells was measured by flow cytometry (8, 11). The kinetic expression patterns of HLA-DR on activated T cells in the MLNC was compatible with the known physiological pattern (16, 17), being highest on day 3 and having progressively decreased by day 5. Thus, the noncancerous cells had no influence on the level of IL-2Rα expression in MLNC. In contrast, CC cells seemed to affect the expression of IL-2Rα by T cells in MLTC. On day 1 post-coculture, the MFI of IL-2Rα expression on activated CD4+ cells was similar in both MLTC and MLNC. It was noteworthy that the MFI of IL-2Rα on CD4+ cells was significantly lower in MLTC than in MLNC on day 3 (170.7 ± 17.0 versus 360.1 ± 14.7) and day 5 (94.1 ± 6.1 versus 174.3 ± 23.6). A similar finding was obtained with CD8+ cells (139.0 ± 8.9 versus 340.0 ± 28.9 on day 3 and 64.2 ± 17.0 versus 196.3 ± 28.7 on day 5 for MLTC and MLNC, respectively; Fig. 1, A and B). However, there was no difference in the kinetic expression patterns of HLA-DR on activated T cells (CD4+ or CD8+) between MLTC and MLNC (Fig. 1, C and D). The expression of HLA-DR on activated T cells of MLTC and MLNC was low on day 1 and elevated on day 3, and remained persistently high on day 5. Our previous study had shown that the expression of HLA-DR, and CD69 as well, on TILs isolated from patients with CC was not altered (8). Together, these findings indicate that CC cells
Next, we studied whether the decreased expression of IL-2Rα in the activated CD8+ T cells was compatible with that in the activated CD4+ T cells. CD8+ T cells were isolated with a purity of >97% from TILs, unstimulated PBMCs, and PHA-activated PBMCs. Expression of IL-2Rα mRNA was measured by competitive RT-PCR. The amount of IL-2Rα mRNA in TIL-derived CD8+ T cells was comparable with that in the activated CD8+ T cells. In both TILs and activated CD8+ T cells, the amount of IL-2Rα mRNA was about 100-fold of that expressed in nonstimulated PBMC-derived CD8+ cells (Fig. 1, E and F). A deficiency in IL-2 protein and IL-2R expression despite adequate levels of IL-2 mRNA has also been shown in TILs from patients with breast cancer (7). Because the IL-2Rα mRNA was abundant in TILs, cancer-mediated suppression did not occur at the transcriptional level.

CC Cells Enhance the Release of sIL-2Rα from Activated T Cells. The possibility exists that the CC-associated decreased expression of IL-2Rα results from excessive shedding of IL-2Rα from T cells. To clarify this possibility, we analyzed the amounts of sIL-2Rα in the supernatants of MLTC and MLNC. The mean concentration of sIL-2Rα in MLNC supernatants was 1045.1 ± 17.3 pg/ml and increased about 2- and 3-fold by day 3 and day 5, respectively. The cumulative sIL-2Rα concentration in the supernatants of MLNC was comparable with the pattern of natural shedding of IL-2Rα (16, 17). However, the mean concentration of sIL-2Rα in the supernatants of MLTC was significantly higher than that in MLNC in day-3 and day-5 cocultures (increased more than 3- and 4-fold, respectively; P < 0.001; Fig. 2A). It became evident that CC cells could enhance the release into the surroundings of sIL-2Rα from activated T cells, and that a proteolytic cleavage might mediate the process (17, 18).

Degraded Expression of IL-2Rα on TILs Is Not Mediated at the Transcriptional Level. Next, we studied whether the decreased expression of IL-2Rα on TILs in CC was mediated at the transcriptional level. CD8+ T cells were isolated with a purity of >97% from TILs, unstimulated PBMCs, and PHA-activated PBMCs. Expression of IL-2Rα mRNA was measured by competitive RT-PCR. The amount of IL-2Rα mRNA in TIL-derived CD8+ T cells was comparable with that in the activated CD8+ T cells. In both TILs and activated CD8+ T cells, the amount of IL-2Rα mRNA was about 100-fold of that expressed in nonstimulated PBMC-derived CD8+ cells (Fig. 1, E and F). A deficiency in IL-2 protein and IL-2R expression despite adequate levels of IL-2 mRNA has also been shown in TILs from patients with breast cancer (7). Because the IL-2Rα mRNA was abundant in TILs, cancer-mediated suppression did not occur at the transcriptional level.

CC Cells Enhance the Release of sIL-2Rα from Activated T Cells. The possibility exists that the CC-associated decreased expression of IL-2Rα results from excessive shedding of IL-2Rα from T cells. To clarify this possibility, we analyzed the amounts of sIL-2Rα in the supernatants of MLTC and MLNC. The mean concentration of sIL-2Rα in MLNC supernatants was 1045.1 ± 17.3 pg/ml and increased about 2- and 3-fold by day 3 and day 5, respectively. The cumulative sIL-2Rα concentration in the supernatants of MLNC was comparable with the pattern of natural shedding of IL-2Rα (16, 17). However, the mean concentration of sIL-2Rα in the supernatants of MLTC was significantly higher than that in MLNC in day-3 and day-5 cocultures (increased more than 3- and 4-fold, respectively; P < 0.001; Fig. 2A). It became evident that CC cells could enhance the release into the surroundings of sIL-2Rα from activated T cells, and that a proteolytic cleavage might mediate the process (17, 18).

CC Cells Enhance the Release of sIL-2Rα from Activated T Cells. The possibility exists that the CC-associated decreased expression of IL-2Rα results from excessive shedding of IL-2Rα from T cells. To clarify this possibility, we analyzed the amounts of sIL-2Rα in the supernatants of MLTC and MLNC. The mean concentration of sIL-2Rα in MLNC supernatants was 1045.1 ± 17.3 pg/ml and increased about 2- and 3-fold by day 3 and day 5, respectively. The cumulative sIL-2Rα concentration in the supernatants of MLNC was comparable with the pattern of natural shedding of IL-2Rα (16, 17). However, the mean concentration of sIL-2Rα in the supernatants of MLTC was significantly higher than that in MLNC in day-3 and day-5 cocultures (increased more than 3- and 4-fold, respectively; P < 0.001; Fig. 2A). It became evident that CC cells could enhance the release into the surroundings of sIL-2Rα from activated T cells, and that a proteolytic cleavage might mediate the process (17, 18).

CC Cells Enhance the Release of sIL-2Rα from Activated T Cells. The possibility exists that the CC-associated decreased expression of IL-2Rα results from excessive shedding of IL-2Rα from T cells. To clarify this possibility, we analyzed the amounts of sIL-2Rα in the supernatants of MLTC and MLNC. The mean concentration of sIL-2Rα in MLNC supernatants was 1045.1 ± 17.3 pg/ml and increased about 2- and 3-fold by day 3 and day 5, respectively. The cumulative sIL-2Rα concentration in the supernatants of MLNC was comparable with the pattern of natural shedding of IL-2Rα (16, 17). However, the mean concentration of sIL-2Rα in the supernatants of MLTC was significantly higher than that in MLNC in day-3 and day-5 cocultures (increased more than 3- and 4-fold, respectively; P < 0.001; Fig. 2A). It became evident that CC cells could enhance the release into the surroundings of sIL-2Rα from activated T cells, and that a proteolytic cleavage might mediate the process (17, 18).

CC Cells Enhance the Release of sIL-2Rα from Activated T Cells. The possibility exists that the CC-associated decreased expression of IL-2Rα results from excessive shedding of IL-2Rα from T cells. To clarify this possibility, we analyzed the amounts of sIL-2Rα in the supernatants of MLTC and MLNC. The mean concentration of sIL-2Rα in MLNC supernatants was 1045.1 ± 17.3 pg/ml and increased about 2- and 3-fold by day 3 and day 5, respectively. The cumulative sIL-2Rα concentration in the supernatants of MLNC was comparable with the pattern of natural shedding of IL-2Rα (16, 17). However, the mean concentration of sIL-2Rα in the supernatants of MLTC was significantly higher than that in MLNC in day-3 and day-5 cocultures (increased more than 3- and 4-fold, respectively; P < 0.001; Fig. 2A). It became evident that CC cells could enhance the release into the surroundings of sIL-2Rα from activated T cells, and that a proteolytic cleavage might mediate the process (17, 18).
MMP Inhibitors Block Cancer-induced Proteolytic Cleavage of IL-2Rα. Using the kinetic experiments of MLTC described above, we conducted a protease inhibition assay to determine whether specific inhibitors could reverse or limit the cancer-induced proteolytic cleavage of IL-2Rα. For achieving maximal expression of IL-2Rα, PBMCs were fully activated with 10 μg/ml PHA for 24 h before MLTC. After 3 days of coculture, the concentration of sIL-2Rα in the MLTC group was ~3-fold that in the T-cells-only group (6001.0 ± 679.6 pg/ml versus 2241.3 ± 197.3 pg/ml; n = 6, P < 0.001). Protease inhibitors such as E-64, aprotinin, bestatin, leupeptin, pepstatin, chymostatin, and antipain-dihydrachloride had no apparent activity in suppressing CC cell-mediated proteolytic cleavage of IL-2Rα (Fig. 2B).

TIMP-2 and MMP-inhibitor I significantly inhibited the CC cell-induced IL-2Rα cleavage in a dose-dependent pattern (Fig. 2B). At a concentration of 1 μg/ml of TIMP-2, cancer-induced IL-2Rα cleavage was totally inhibited. The concentration of sIL-2Rα in the TIMP-2-MLTC group was 1953.1 ± 81.2 pg/ml, compared with a concentration of 6001.0 ± 679.6 pg/ml in the MLTC group without inhibitor (n = 6; P = 0.004). At a concentration of 0.1 μg/ml TIMP-2, partial inhibition existed (3136.2 ± 327.7 pg/ml versus 6001.0 ± 679.6 pg/ml; P = 0.011). At a concentration of 100 μM/ml MMP-inhibitor I, IL-2Rα cleavage was also totally inhibited. The concentration of sIL-2Rα in the MMP-inhibitor I-MLTC group was 2114.7 ± 165.7 pg/ml compared with a concentration of 6660.1 ± 640.5 pg/ml in the MLTC group without inhibitor (n = 6; P = 0.001). At a concentration of 10 μM/ml of MMP-inhibitor I, partial inhibition existed (3264.2 ± 226.2 pg/ml versus 6660.1 ± 640.5 pg/ml; P = 0.006). The finding indicates that an MMP-mediated proteolytic process is likely to be responsible for the IL-2Rα cleavage, and that MMP inhibitors block this process.

MMP Inhibitors Restore IL-2-promoted Proliferation Function of TILs. To examine whether TIMP-2 or MMP-inhibitor I added to cultures could restore the proliferative function of cancer-encountered T cells, we also performed an IL-2-promoted T-cell proliferation assay. In the MLTC experiments illustrated in Fig. 2C, cancer-encountered T cells proliferated poorly in the presence of IL-2. However, at a concentration of 1 μg/ml TIMP-2 or 100 μM/MMP-inhibitor I, the proliferative ability of cancer-encountered T cells could be restored in an IL-2 dose-dependent manner, which indicated that MMP inhibitors are functionally capable of restoration of the T-cell proliferation function by blocking the cancer-induced IL-2Rα cleavage.

MMP-1, -2, and -9 Are Abundantly Expressed by CC Cells. We then studied the expression of various types of MMPs, including MMP-1, -2, -3, -7, -8, -9, and -13, two membrane-type MMPs (MT1 and MT2), and three types of inhibitors (TIMP-1, -2, and -3) by immunohistochemical staining in 30 cases of CC tissues. MMP-1, MMP-2, and MMP-9 are abundantly expressed in most, if not all cancer cells, but are not expressed or are only very weakly expressed in normal cervical epithelial or stromal cells (Fig. 3, A–D, data not shown). The expression of other types of MMPs and two types of membranous MMPs was not detected or was only weakly expressed. Diffusely weak stainings of TIMP-1, TIMP-2, and TIMP-3 were observed in selected cases of CC (weakly expressed in 10–20% of cases). Low TIMP expression and/or increased MMP:TIMP ratio in tissues were reported to associate with a poor prognosis for CC (19, 20). In the gelatin zymographic study (Fig. 3E), the gelatinolytic activities of MMP-9 (Mr 92,000) were strongly expressed in all CC cells but varied widely in supernatants of MLTC (data not shown). The enzyme activities of MMP-9 in activated T cells were about 1–8% of CC cells.

MMPs Directly Mediate the Proteolytic Cleavage of IL-2Rα. To characterize further the role of MMP in IL-2Rα cleavage, we directly incubated activated T cells with 1 μg/ml recombinant MMP-1, MMP-2, or MMP-9 protease. As demonstrated by flow-cytometric analysis, MMP-1 had no effect on the expression of IL-2Rα on activated T cells (Fig. 4A). MMP-9 and, to a lesser extent, MMP-2, can down-regulate the expression of IL-2Rα on activated T cells (Fig. 4, B and C). In the experiments illustrated in Fig. 4D, MMP-2-treated T cells proliferated well in an IL-2 dose-dependent manner, which was compatible with the pattern of activated T-cell controls. MMP-9-treated T cells proliferated poorly in the presence of low-dose IL-2, but their proliferative ability could be restored by either IL-2 at a higher dose or MMP-inhibitors. Thus, the observed cancer-mediated IL-2Rα down-regulation can be attributed mainly to proteolytic cleavage by MMP-9 from cancer cells. Because IL-2Rα plays a pivotal role in the development and propagation of functional T cells, cleavage of IL-2Rα down-regulates the proliferative capability of T cells (16, 17; Figs. 2C and 4D).

The general roles of extracellular-matrix enzymes, especially MMPs, and the immunological reaction remain to be investigated (21). The MMPs have different substrate specificities with complicated regulation of cytokines and other inflammatory mediators on immune cells (21–23). Changes in the balance between active MMPs and their inhibitors may result in a number of human disorders, including inflammation, fibrosis, and tumor invasion (21–26). The latent pro-MMPs permit autocatalytic cleavage and MMP-activating proteolytic pathways to generate the fully active MMPs. However,
Activated T cells were used as controls. Data from four independent experiments are presented as mean ± SE. A significant left shift of IL-2R expression on activated T cells by MMPs. A, representative histograms of IL-2Rα expression on MMP-1-treated (solid curve) and -nontreated (dotted curve) activated T cells. B, representative histograms of IL-2Rα expression on MMP-2-treated (solid curve) and -nontreated (dotted curve) activated T cells. C, representative histograms of IL-2Rα expression on MMP-9-treated (solid curve) and -nontreated (dotted curve) activated T cells. D, IL-2-promoted [3H]Tdr proliferation assays of MMP-treated T cells. Various amounts of IL-2 were added to the cultures of purified TILs within the tumor milieu.

It is less known about the molecular mechanisms that regulate the augmented proteolytic cascades of pro-MMPs in immune cells (21–24). In macrophages and T cells, MMP-2 and MMP-9 can be expressed (22, 23), and the expression of MMP-9 in T cells was dependent on the activation status of these T cells and was regulated by IL-2 (27). Expression of MMP is important for the trans-basement-membrane migration of T cells and for MMP-activating proteolytic cleavage of certain cytokines and receptors (21–26).

MMPs are abundant in a variety of human cancer cells, including those of CC (19, 20, 25). Cancer-derived MMPs may trigger the proteolytic cleavage of cytokines and their receptors, including tumor necrosis factor-R (28, 29), IL-6R (29, 30), and also IL-2Ra, as shown in the present study. A recent study by Wang et al. (31) showing that MMP-9-deficient mice cannot resolve a contact hypersensitivity reaction further implies the down-regulatory role of MMP-9 in immune responses. Moreover, cancer-encountered T cells lacking IL-2Ra signaling may be resistant to Fas-mediated apoptosis (32, 33), which may reciprocally explain the abundance of, but the relatively poor function of, TILs within the tumor milieu.

In conclusion, the mechanism for apparent inhibition of IL-2Ra expression on activated T cells in tumors is governed by a cancer cell-directed and MMP-dependent cleavage. Cancer-derived MMP-9, and other MMPs as well, can inhibit the proliferative function of tumor-encountered T cells, and this may explain the relative anergic state of TILs. Through the MMP-proteolytic pathway, cancer cells may facilitate immuneescape, cancer invasion, and metastasis. It is noteworthy that TIMP activity was significantly lower in CC tissues than in other gynecological cancer tissues (20), and that a reduction in TIMP expression in tissue is associated with a poor prognosis for CC (19). Reversal of tumor-induced immunosuppression has been a major concern regarding prospects for adoptive T-cell therapy. Our finding should provide important insights into the understanding of the interaction between cancer cells and the immune system, as well as into the development of anticancer strategies.

Fig. 4. Cleavage of surface IL-2Ra on activated T cells by MMPs. A, representative histograms of IL-2Ra expression on MMP-1-treated (solid curve) and -nontreated (dotted curve) activated T cells. B, representative histograms of IL-2Ra expression on MMP-2-treated (solid curve) and -nontreated (dotted curve) activated T cells. C, representative histograms of IL-2Ra expression on MMP-9-treated (solid curve) and -nontreated (dotted curve) activated T cells. D, IL-2-promoted [3H]Tdr proliferation assays of MMP-treated T cells. Various amounts of IL-2 were added to the cultures of purified activated T cells in the presence of MMP-2 (○), MMP-9 (●), and MMP-9+NIMP (▲). Activated T cells were used as controls (□). Data from four independent experiments are presented as mean ± SE.

REFERENCES


A Novel Role of Metalloproteinase in Cancer-mediated Immunosuppression

Bor-Ching Sheu, Su-Ming Hsu, Hong-Nerng Ho, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/1/237

Cited articles
This article cites 31 articles, 11 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/1/237.full#ref-list-1

Citing articles
This article has been cited by 32 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/1/237.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.