Brief Definitive Report

EXPRESSION AND ROLE OF p75 INTERLEUKIN 2 RECEPTOR ON HUMAN MONOCYTES

By IGOR ESPINOZA-DELGADO,* JOHN R. ORTALDO,* ROBIN WINKLER-PICKETT,* KAZUO SUGAMURA,‡ LUIGI VARESIO,* AND DAN L. LONGO*

*From the *Biological Response Modifiers Program, National Cancer Institute, Frederick, Maryland 21701; and the ‡Department of Bacteriology, Tohoku University, School of Medicine, Sendai 980 Japan*

IL-2, initially discovered for its mitogenic activity on T cells (1), also acts on monocytes (2). T and B cells express at least two forms of IL-2 receptor (IL-2R): the p55 glycoprotein (IL-2Rα), which is recognized by the anti-Tac mAb (3), and the p70–75 kD glycoprotein (IL-2Rβ), which reacts with the TU27 mAb (4). The p55 chain binds IL-2 with a low affinity ($K_d \sim 10^{-8}$ M), the p70–75 chain binds IL-2 with an intermediate affinity ($K_d \sim 10^{-9}$ M), and both molecules form a noncovalently linked heterodimer that binds IL-2 with high affinity ($K_d \sim 10^{-12}$ M). The response of T or B cells to IL-2 seems to be mediated by p70–75 and not p55 (5, 6). Human monocytes respond to IL-2 with induction of IL-1 mRNA (7), development of tumoricidal activity (2), and we have recently shown that IL-2 enhances c-fms mRNA expression (Espinoza-Delgado et al., manuscript submitted). The ability of human monocytes to respond to IL-2 indicates the presence of a functional receptor for IL-2 on these cells. However, fresh human monocytes do not express Tac antigen (8), and there are no reports concerning the expression of p70–75 on fresh or IL-2-treated monocytes. We have investigated the surface expression of p70–75, and its potential role in the cytotoxic response of monocytes to IL-2, using the anti-p75 mAb TU27 (4). We demonstrated that human monocytes express constitutively high levels of p75 and that TU27 mAb blocks the activation to a cytolytic stage induced by IL-2 but does not block IFN-γ–induced, monocyte-mediated cytolysis.

Materials and Methods

Cell Culture. Monocytes were purified from leukophoresed PBMC by centrifugal elutriation (9). The purity of the monocyte preparation used in these studies was 93 ± 3% as assessed by morphology on Giemsa-stained cytocentrifuge preparations and flow cytometry using the monocyte-specific mAb Leu M3. Cells were cultured in RPMI 1640 (Advanced Biotechnology, Inc., Columbia, MD), containing penicillin 100 U/ml, streptomycin 100 μg/ml, 2 mM L-glutamine, 20 mM Hepes (Gibco Laboratories, Grand Island, NY) and 10% heat-inactivated FCS (HyClone Laboratories, Logan, UT). Recombinant human IL-2 was kindly provided by Cetus Corp., Emeryville, CA. Recombinant human IFN-γ was kindly provided by Genentech, Inc., South San Francisco, CA. LPS levels were less than 12 pg/ml in all reagents and media used.

Address correspondence to Dr. Igor Espinoza-Delgado, Laboratory of Molecular Immunoregulation, National Cancer Institute, Building 560, Room 21-89A, Frederick, MD 21701-1013.

Monoclonal Antibodies. Anti-Tac mAb was provided by Dr. T. Waldmann, NIH, Bethesda, MD. Anti-p75 (TU27) mAb was provided by Dr. T. Takeshita, Tohoka University School of Medicine, Sendai, Japan. Antimonocyte differentiation antigen Leu M3 mAb and anti-T cell CD3 mAb were purchased from commercial sources (Becton Dickinson & Co., Mountain View, CA). Anti-IFN-γ mAb mouse IgG1 (k) (1×10^6 IFNγ NU/mg) was provided by the Biological Resources Branch, BRMP (10).

Cytotoxicity. Monocytes were cultured under nonadherent conditions in 96-well round-bottomed polypropylene microtiter plates (Wilks Precision Instruments, Rockville, MD). IL-2 or IFN-γ was added into test wells containing monocytes for 24 h and then washed extensively before adding labeled tumor target cells. HT29, a human colon carcinoma cell line (American Type Culture Collection, Rockville, MD; IL-2 units used in this paper are Cetus units, 1 Cetus unit is equivalent to 6 international units), was used as a target. HT29 cells were labeled by incubating 5×10^6 tumor cells with 40 μCi ^{111}Indium (^{111}In) (Amersham Corp., Arlington Heights, IL) for 15 min at room temperature in 1 ml of medium. Triplicate monocyte cultures were incubated with 5×10^3 labeled target cells at an E/T ratio of 20:1 in 200 μl of medium for 48 h. The plates were then centrifuged for 5 min at 350 *g*, and 75 μl of the culture supernatant were harvested and the radioactivity was measured. The results were expressed as percentage of ^{111}In released into the medium calculated from the average cpm of triplicate samples as [(experimental cpm − spontaneous cpm)/(total cpm − spontaneous cpm)] × 100.

Flow Cytometry. Cells were treated with FITC- or phycoerythrin (PE)-labeled mAbs (Becton Dickinson & Co., Mountain View, CA) according to the following procedures: 5×10^5 cells were incubated with 20 μl of the appropriate dilution of mAb in 0.1 ml of PBS supplemented with 2% human AB serum and 0.1% sodium azide (Gibco) for 30 min at 4°C. Samples of 20,000 cells were analyzed on a FACScan Flow Cytometer.

Results

The expression of p55 and p75 proteins in fresh human monocytes (92% Leu M3$^+$, CD3$^-$; range 90–96% [Fig. 1 *B*]) from 10 different donors was studied by two-color flow cytometry. When these cells were stained with FITC-TU27 and Leu M3-PE mAbs (Fig. 1 *C*), nearly all the Leu M3$^+$ cells (93%) expressed p75 protein. In contrast, when the monocytes were stained with FITC-labeled anti-Tac and Leu M3-PE (Fig. 1 *D*), none of the monocytes expressed detectable p55 protein.

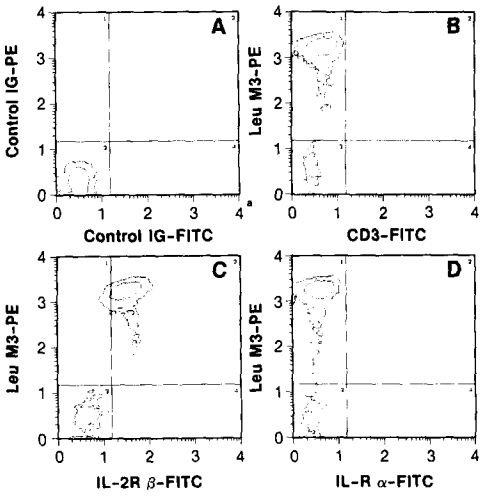

FIGURE 1. Expression of IL-2Rβ and IL-2Rα on fresh monocytes. Monocytes were stained with FITC- or PE-conjugated mAbs. Contour plots of monocytes stained with (*A*) FITC-conjugated IgG1 control mAb and PE-conjugated IgG2b control mAb. (*B*) CD3-FITC and Leu M3-PE mAbs; (*C*) FITC-TU27 (IgG1) and Leu M3-PE mAbs; (*D*) FITC-anti-TaC (IgG2a) and Leu M3-PE mAbs. (*a*) Decades of a fluorescence logarithmic scale.

Experiments were performed to determine whether the expression of p75 was modulated during the activation of monocytes to a tumoricidal stage by IFN-γ or IL-2. Monocytes were cultured for 24 h in medium alone or supplemented with 1,000 U/ml of IL-2 or 500 U/ml of IFN-γ and tested for p55 and p75 expression. Fig. 2 shows the Leu M3$^+$, CD3$^-$ gated flow cytometry histograms of the various monocyte populations. The results (Fig. 2 *A*) demonstrated that the expression of p75 by monocytes is not influenced by treatment with IL-2. In contrast, the same monocytes respond to IFN-γ but not to IL-2, with a major increase in percentage of p55-expressing cells (Fig. 2 *B*).

To study the role of p55 and p75 in the activation of monocytes by IL-2, we examined the effects of TU27 or anti-Tac mAbs in the expression of cytotoxic activity. IFN-γ-activated monocytes were used as a specificity control. Under the experimental conditions used, IL-2 or IFN-γ alone induced cytotoxic activity in human monocytes. Dose-response experiments demonstrated that 1,000 U/ml of IL-2 or 500 U/ml of IFN-γ induced plateau levels of cytotoxicity (data not shown), and these concentrations were used in the following experiments. Cells were incubated for 24 h with increasing concentrations of TU27 or anti-Tac mAbs with or without IL-2 or IFN-γ; cells were then washed and assayed for cytotoxic activity. Depicted in Fig. 3 *A* is the result of a representative experiment. IL-2-treated monocytes expressed cytotoxic activity (38%) that was not affected by the addition of increasing amounts of anti-Tac mAb. In contrast, TU27 mAb caused a dose-related decrease of IL-2-induced

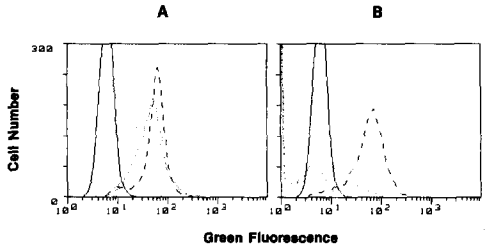

FIGURE 2. Expression of IL-2Rβ and IL-2Rα on monocytes activated by IL-2 or IFN-γ. Monocytes were treated with 1,000 U/ml of IL-2 or 500 U/ml of IFN-γ for 24 h before staining with mAb. Histograms of Leu3$^+$ monocytes stained with FITC-TU27 (*A*) or with FITC-anti-Tac mAb (*B*). Medium (· · ·) IL-2 treated with monocytes (·····). IFN-γ-treated monocytes (---), relative to CD3-FITC control antibody (——).

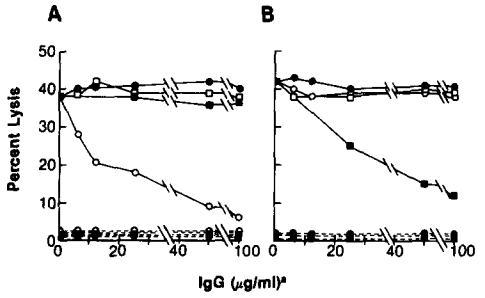

FIGURE 3. Effects of mAbs on the activation of monocytes by IL-2 or IFN-γ. (*A*) Elutriated monocytes were cultured with medium (---) or 1,000 U/ml of IL-2 (——). (*B*) Monocytes were cultured with medium (---) or 500 U/ml of IFN-γ (——). TU27 mAb (○), isotype-matched control Ig (●), anti-Tac mAb (□), anti-IFN-γ mAb (■). (*a*) Relative concentration of mAbs. 100 is equal to 100 μg/ml of TU27, isotype-matched IgG, anti-Tac, or 2,000 neutralizing U/ml of anti-IFN-γ mAbs. Monocytes were preincubated with TU27, anti-Tac or isotype-matched Ig mAbs for 1 h at 37°C and then activators were added. The spontaneous release of ^{111}In from target cells cultured alone was <10% of the total incorporated radioactivity. All values represent the means of triplicate determinations. The standard errors were <10% of the mean.

cytotoxic activity that reached maximum at concentrations of TU27 of 50 μg/ml. TU27 mAb alone did not activate monocytes. Anti-IFN-γ mAb used as a control did not affect the induction of cytotoxic activity by IL-2. These results demonstrate that anti-p75 but not anti-p55 mAbs block the activation of monocytes by IL-2. To test whether the inhibitory effects of TU27 were selective for IL-2-induced cytotoxicity, experiments were done with monocytes activated with IFN-γ (Fig. 3 *B*). TU27 did not affect the activation of monocytes by IFN-γ. Under the same conditions anti-IFN-γ mAb at concentrations of 1,000 neutralizing U/ml, substantially decreased the ability of IFN-γ to induce cytotoxicity. These results demonstrate that the inhibitory effects of TU27 are restricted to the IL-2-induced monocyte activation.

Discussion

Understanding of the mechanisms by which IL-2 activates monocytes has been hampered by the lack of information concerning the expression of IL-2R proteins on these cells. Although p55 can be induced by treatment of monocytes with IFN-γ (8), we have found that Tac antigen is not expressed on fresh human monocytes, confirming and extending previous reports. Moreover, we have observed that anti-Tac mAb does not affect the expression of tumoricidal activity by IL-2-activated monocytes. Overall these results indicate that the Tac antigen may not be involved in the activation of monocytes by IL-2. Using the mAb TU27 that specifically recognizes p75 (4), we demonstrated that a high percentage of fresh resting human monocytes expressed p75 protein. To date monocytes from 10 different normal donors have been tested for p75 expression and more than 90% of such monocytes react with TU27 in all donors tested.

To examine the role of p75 in human monocyte activation, we investigated the effects of TU27 mAb on the cytotoxic activity of monocytes induced by IL-2. TU27 inhibited in a dose-dependent manner the activation of monocytes to a tumoricidal stage by IL-2. The inhibitory effects of TU27 were not due to a toxic effect since that mAb did not affect IFN-γ-induced cytotoxicity. Moreover, isotype-matched IgG did not inhibit the activation of monocytes by IFN-γ or IL-2. Conversely, neutralizing antibodies to IFN-γ blocked the activation of monocytes by IFN-γ but not by IL-2. These results indicate that the activation of monocytes cannot be accounted for by minute amounts of IFN-γ that may be released in the medium by the few nonmonocytic contaminating cells present in the culture. This conclusion is strengthened by the observation that IFN-γ was never detected by RIA in the supernatant of IL-2-treated monocytes (data not shown). In conclusion, these results argue against the possibility that the inhibitory effects of TU27 on monocyte activation by IL-2 may be due to a block of IL-2-induced IFN-γ release by contaminating lymphocytes. In contrast, our results support the idea that TU27 blocked the biological effects of IL-2 by interfering with the function of p75. Significant inhibition of the cytotoxic activity requires more than 10 μg/ml of TU27, a concentration 10 times higher than that required to saturate the receptor. The differences may be due to internalization and recycling of IL-2Rs occurring during the activation but absent during FACS studies performed at 4°C in the presence of NaN_3.

The demonstration of p75 on resting monocytes provides an explanation for the ability of these cells to respond to IL-2. Constitutive expression of p75 has been detected on $CD3^-$ LGL (NK cells) and it has been shown that the augmentation of

the cytolytic activity of NK cells and LAK cells by IL-2 can be blocked by TU27 antibodies (11; and Ortaldo, J. R., et al., manuscript submitted for publication). These results indicate that natural effector cells that respond to IL-2 with antitumor activity and cytokine secretion share the constitutive expression of p75, which may be important for the prompt activation of these effector cells during an immune response. However, the cellular events that follow the stimulation of the p75 receptor-bearing monocytes by IL-2 are different from the events that follow IL-2 stimulation of p75 receptor-bearing LGL. IL-2 is mitogenic for LGL but not for monocytes. Thus, the comparison of the molecular events elicited by IL-2 in $CD3^-$ LGL and monocytes may provide important insights on differences in IL-2R biology in distinct cell lineages.

Summary

We investigated the expression of IL-2R subunits in human monocytes using the TU27 mAb, which recognizes the p75 chain, and anti-Tac mAb, which recognizes the p55 moiety of the IL-2R. We found that p75 but not p55 is constitutively expressed in more than 90% of fresh human monocytes. Antibody to p75, but not to p55, inhibited the activation of monocytes to a cytotoxic stage induced by IL-2 but did not block IFN-γ-induced cytotoxicity. Our data demonstrate that the p75 chain is expressed on human monocytes and is involved in the activation of monocytes by IL-2.

We wish to thank Dr. Joost J. Oppenheim and Dr. George W. Cox for their critical comments, and Ms. Bobbie Unger for her editorial assistance.

Received for publication 20 November 1989 and in revised form 12 February 1990.

References

1. Morgan, D. A., F. W. Ruscetti, and R. C. Gallo. 1976. Selective in vitro growth of T lymphocytes from normal human bone marrows. *Science (Wash. DC).* 193:1007.
2. Malkovsky, M., B. Loveland, M. North, G. L. Asherson, L. Gao, P. Ward, and W. Fiers. 1987. Recombinant interleukin 2 directly augments the cytotoxicity of human monocytes. *Nature (Lond.).* 325:262.
3. Waldmann, T. A. 1986. The structure, function and expression of interleukin 2 receptors on normal and malignant lymphocytes. *Science (Wash. DC).* 232:727.
4. Takeshita, T., Y. Goto, K. Tada, K. Nagata, H. Asao, and K. Sugamura. 1989. Monoclonal antibody defining a molecule possibly identical to the p75 subunit of interleukin 2 receptor. *J. Exp. Med.* 169:1323.
5. Ferris, D. K., J. Willette-Brown, J. R. Ortaldo, and W. L. Farrar. 1989. IL-2 regulation of tyrosine kinase activity is mediated through the p70-75 β-subunit of the IL-2 receptor. *J. Immunol.* 143:870.
6. Tigges, M. A., L. S. Casey, and M. E. Koshland. 1989. Mechanism of interleukin-2 signaling: mediation of different outcomes by a single receptor and transduction pathway. *Science (Wash. DC).* 243:781.
7. Kovacs, E. J., S. K. Beckner, D. L. Longo, L. Varesio, and H. A. Young. 1989. Cytokine gene expression during the generation of human lymphokine-activated killer cells: early induction of interleukin 1β by interleukin 2. *Cancer Res.* 49:940.
8. Herrmann, F., S. A. Cannistra, H. Levine, and J. D. Griffin. 1985. Expression of interleukin 2 receptor and binding of interleukin 2 by gamma interferon-induced human leu-

kemic cells and normal monocytic cells. *J. Exp. Med.* 162:1111.
9. Stevenson, H. C. 1984. Isolation of human mononuclear leukocyte subsets by counter current centrifugal elutriation. *Methods Enzymol.* 108:242.
10. Wang, C. Y., Y. Bushkin, P.-D. Chen, C. D. Platsoucas, and C. Long. 1984. Preparation and characterization of monoclonal antibodies directed at epitopes of human IFNγ. *Hybridoma.* 3:321.
11. Phillips, J. H., T. Takeshita, K. Sugamura, and L. L. Lanier. 1989. Activation of natural killer cells via and p75 interleukin 2 receptor. *J. Exp. Med.* 170:291.