The lymphocytosis manifested in infectious mononucleosis (IM) during acute phase is ascribed to a reactive expansion of CD8+ T lymphocytes caused by Epstein-Barr virus (EBV)-infected B lymphocytes. Expression of HLA-DR antigen on IM lymphocytes suggests that these T lymphocytes are somehow activated in vivo. In the present study, we analyzed the Interleukin-2 (IL-2) receptor expression on lymphocytes from six patients with acute IM. Radioisotope-labeled IL-2 binding assay revealed that IM lymphocytes from all patients examined had a considerable number of IL-2 binding sites with an intermediate affinity, although they did not express the IL-2 receptor recognized by anti-Tac antibody (p55). The number of binding sites (1,070 to 4,600 sites per cell) was larger than that of a normal, resting T lymphocyte-enriched population (660 sites per cell). Furthermore, IM lymphocytes showed marked proliferative responses to higher concentrations of IL-2, which were almost completely blocked by an anti-p70 IL-2 receptor antibody, indicating that their IL-2 receptor is a functional receptor. The results of an affinity cross-linking study seem to indicate that the IL-2 receptor expressed on IM lymphocytes is p70, the second chain of the IL-2 receptor distinct from p55. Flow cytometric analysis following immunofluorescent staining with anti-p70 IL-2 receptor antibody confirmed p70 expression on CD8+ HLA-DR+ lymphocytes. These data suggest that p70 IL-2 receptor expression is involved in the immune response triggered by EBV infection. © 1990 by The American Society of Hematology.

Infectious mononucleosis (IM) is an acute and usually self-limiting disease produced by primary infection with Epstein-Barr virus (EBV). It is manifested hematologically by an increase in lymphocytes and the occurrence of atypical lymphocytes. Immunologic studies have demonstrated that the majority of expanded lymphocytes are not EBV+ B lymphocytes but CD8+ T lymphocytes. Peripheral blood lymphocytes from patients with IM during acute phase include an increased number of HLA-DR+ T lymphocytes and exhibit "non-HLA restricted" cytotoxic activity against a series of EBV+ target cells, suggesting that these cells are somehow activated in vivo. However, the mechanism of their activation and proliferation is still unclear. While it is accepted that most T lymphocytes, when stimulated by antigens or lectins in vitro, express the Interleukin-2 (IL-2) receptor/Tac antigen (p55), IL-2 receptors from IM patients do not express Tac antigen. Recent evidence has indicated that non-Tac IL-2 binding peptide (p70), which constitutes the high affinity IL-2 receptor in association with p55, can exist without p55 and may play a role as a functional receptor. Freshly isolated, large granular lymphocytes are reported to express a larger quantity of p70 than small, resting T lymphocytes, both of which do not express p55. However, little is known about the regulation of expression of p70; namely, whether it is inducible on the occasion of T lymphocyte activation. In the present report, we examined p70 IL-2 receptor expression on lymphocytes from IM patients and discussed the activated state of T lymphocytes without Tac expression.

MATERIALS AND METHODS

Patients. Six patients with IM during acute phase were examined. The diagnosis was based on common clinical symptoms of acute IM, atypical lymphocytes in the peripheral blood, and the cell surface phenotype of lymphocytes. All patients were positive for heterophile antibody. Blood samples used in this study were taken within 2 weeks of the onset and the white blood cell (WBC) counts were 10,000 to 27,300/μL.

Cell separation. Peripheral blood mononuclear cells (PBMCs) were separated from heparinized venous blood by Ficoll-Conray density gradient centrifugation. The collected interface cells were washed twice with phosphate-buffered saline (PBS) and once with RPMI 1640 medium supplemented with 10% fetal calf serum (FCS; MA Bioproducts, Walkersville, MD; growth medium). For the control, we used fresh PBMC or a T lymphocyte-enriched population from normal volunteers. PBMC from normal volunteers were passed over a nylon-wool column to yield a T lymphocyte-enriched population. This preparation contained more than 95% CD2+ cells and less than 2% CD14+ cells (monocytes) or CD20+ cells (B lymphocytes) determined by cytofluorometry. In the experiments of IL-2 response, we also prepared normal phytohemagglutinin (PHA)-P-stimulated lymphocytes (PHA-blasts) for the control as described.

Cell surface marker analysis. Monoclonal antibodies (MoAbs) OKT3 (CD3), OKT4 (CD4), OKT8 (CD8), OKT11 (CD2), and OKIa1 (HLA-DR) were obtained from Ortho Pharmaceutical Corp (Raritan, NJ). Leu-2a and Leu-3a MoAbs were obtained from Beckton Dickinson Immunocytometry Systems (Mountain View, CA). Anti-Tac and 7G7/B6 (kindly provided by Dr D. L. Nelson, National Institute of Health, Bethesda, MD) were used as anti-human IL-2 receptor (p55) antibodies. In three cases, we used 2R-B antibody, an anti-p70 IL-2 receptor antibody that we have recently developed. MoAb 2R-B is considered to be able to recognize p70 human IL-2 receptor because of the following data: (1) MoAb 2R-B inhibited IL-2 binding to YT-2C2 cells expressing only p70 but not to MT-1 cells expressing only p55 IL-2 receptor; (2) immunoprecipitation followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis revealed that 2R-B antibody was directed against a 70-kDa protein. The purified 2R-B antibody was used to identify p70-expressing lymphocytes. The purified 2R-B antibody was used to identify p70-expressing lymphocytes. The purified 2R-B antibody was used to identify p70-expressing lymphocytes. The purified 2R-B antibody was used to identify p70-expressing lymphocytes.

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Submitted December 19, 1988; accepted September 6, 1989.
Supported by Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

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reacts with p70 to p75; (3) radiolabeled IL-2 binding assay in the presence of 2R-B antibody at 4°C showed the disappearance of high but not low affinity receptors; (4) large granular lymphocyte leukemia cells that express intermediate affinity receptors reacted with 2R-B antibody but not with anti-Tac antibody; and (5) 2R-B antibody and Mik J1 antibody\(^2\) mutually inhibit binding to YT-2C2 cells. Surface antigenic targets were detected by direct or indirect immunofluorescence and cytometry using Spectrum (Ortho Diagnostics, Westwood, MA) or FACScan (Beckton Dickinson) as described.\(^2\)

Radiolabeled IL-2 binding assay. Human recombinant IL-2 (kindly provided by Takeda Chemical Industries Inc, Osaka, Japan) was radiolabeled with Na\(^{251}\)I\(^2\) (16.9 mCi/μg of iodine, Amersham International, Buckinghamshire, UK) by the chloramine T method. The specific radioactivity was 17,000 to 22,000 cpm/μg. A total of 2 \(\times 10^6\) cells in RPMI 1640 medium containing 25 mmol/L HEPES (pH 7.4), 10 mg/mL bovine serum albumin, and 1 mg/mL sodium azide was incubated with serial dilutions of \(^{125}\)I-labeled IL-2 on ice for 1 hour and then centrifuged through a layer of mixture of 20% olive oil and 80% di-n-butyl phthalate. The tips of the tubes containing the cell pellet were cut off, and the radioactivity was counted in a gamma counter. Non-specific binding was determined by adding 250-fold excess amount of unlabeled IL-2. The number of IL-2 binding sites and dissociation constant (kd) were calculated by Scatchard analysis.

Proliferative response of IM lymphocytes to IL-2. Proliferation of lymphocytes from IM patients in response to IL-2 was measured by \(^3\)H[TdR] incorporation as described.\(^2\) Briefly, \(1 \times 10^6\) cells in a total volume of 200 μL of the growth medium were incubated in a 96-well flat bottom plate in the presence of serial dilutions of IL-2 for 72 hours. For the inhibition study, an excess amount of purified anti-Tac antibody (630 nmol/L), 2R-B antibody (630 nmol/L), or both antibodies was added to the culture. We used anti-Lyt 2, 1 MoAb (IgG2a) and X63 MoAb (IgG1) as controls. Cells were pulsed with 0.5 μCi/well \(^3\)H-TdR (Amersham) for the last 6 hours and harvested onto glass fiber filters. The radioactivity was counted by liquid scintillation.

Affinity labeling of the IL-2 receptor on IM lymphocytes. Affinity labeling of the IL-2 receptor was performed as described previously.\(^2\) Briefly, after the incubation with 5 mmol/L \(^{125}\)I-labeled IL-2 on ice for 1 hour, 5 \(\times 10^6\) cells were treated with 100 μg/mL disuccinimidyl suberate (Nakarai Chemicals, Kyoto, Japan) for 20 minutes in PBS, pH 8.3, containing 1 mmol/L MgCl\(_2\). The reaction was quenched with 10 mmol/L Tris, pH 8.3, 1 mmol/L EDTA, and 0.14 mol/L NaCl. Cells were pelleted, extracted and then analyzed by SDS-PAGE as described.\(^2\)

**RESULTS**

**Cell surface marker.** The cell surface phenotypes of PBMC from the IM patients examined are shown in Table 1. They contained more than 74% CD8\(^+\) cells and more than 73% HLA-DR\(^+\) cells, indicating that not only B lymphocytes but also the majority of expanded CD8\(^+\) T lymphocytes expressed HLA-DR antigen. As reported previously,\(^1\) no significant expression of Tac antigen (p55) was detected in all six patients. PBMC from three patients were tested for reactivity with 2R-B antibody, an anti-p70 IL-2 receptor antibody. MoAb 2R-B reacted with 77%, 89%, and 94% of PBMC from patients 4, 5, and 6, respectively. Dual staining using anti-CD8 antibody and 2R-B antibody confirmed the p70 IL-2 receptor expression on the majority of CD8\(^+\) lymphocytes (Fig 1).

**Radiolabeled IL-2 binding assay.** Because PBMC from IM patients were negative for Tac antigen, we performed radiolabeled IL-2 binding assay to examine whether these cells have some IL-2 binding sites distinct from the Tac peptide (p55). A Scatchard plot of the representative binding experiment in patient 6 is shown in Fig 2. As we had expected, IM lymphocytes expressed a certain number of IL-2 binding sites with a single affinity. The cells from the six patients had 1,070 to 4,600 IL-2 binding sites per cell, and the affinity seemed to be intermediate (kd = 0.7 to 2.6 mmol/L; Table 2). Normal resting T lymphocyte-enriched populations expressed fewer IL-2 binding sites (650 sites/cell) with an intermediate affinity.

**Proliferative response of IM lymphocytes to IL-2.** To learn the signaling capability of the non-Tac IL-2 receptor expressed on IM lymphocytes, we examined the proliferation of these cells in response to IL-2. As shown in Fig 3, PBMC from all patients examined exhibited marked proliferative responses. Proliferative responses to IL-2 were comparable with those of normal PHA-blasts in cases 2, 3, and 4. Although the maximal \(^3\)H-TdR uptakes were less in the remaining three cases, lymphocytes from these patients clearly proliferated in response to added IL-2. Normal fresh PBMC or T lymphocyte-enriched population also responded, but very weakly.

Then we examined the effect of anti-p55 (Tac) IL-2 receptor antibody and anti-p70 IL-2 receptor antibody (2R-B antibody) on IL-2--induced proliferative responses of IM lymphocytes. Figures 4A, B, and C show the representative
results of the studies of cases 4, 5, and 6. PBMC from three patients proliferated well in response to optimal doses of IL-2. Control antibody (anti-Lyt-2.1) did not show any effect. MoAb 2R-B, as well as the combination of 2R-B antibody and anti-Tac antibody, almost completely inhibited cell proliferation induced by optimal doses (32 to 2,000 pmol/L) of IL-2, indicating that p70 IL-2 receptor is involved in these proliferative responses. Addition of higher doses (8 to 32 nmol/L) of IL-2 abolished the inhibitory effect of 630 nmol/L 2R-B antibody in cases 5 and 6. The effect of anti-Tac (p55) antibody was variable. In case 6, in which the proportion of CD3+, CD8+ HLA-DR+ 2R-B+ lymphocytes was markedly high, the proliferative response of PBMC to IL-2 was not inhibited by anti-Tac antibody at any IL-2 concentrations examined. In case 5, in which the proportion of CD8+ lymphocytes was not markedly high, anti-Tac antibody considerably inhibited IL-2-induced proliferative response. In case 4, responses were slightly inhibited by anti-Tac antibody.

Affinity cross-linking of IL-2 receptor expressed on IM lymphocytes. To clarify the relation between the p70 and the IL-2 binding components on IM lymphocytes, affinity cross-linking studies were performed in IM lymphocytes and normal PBMC cultured with 0.1% PHA-P for 3 days (PHA-blasts; Fig 5). In PHA-blasts, two major bands with an apparent mol wt of 70,000 to 75,000 and 85,000 to 90,000 were detected, the larger of which ran as a doublet. These two bands are considered to represent the p55 and the p70 cross-linked with IL-2, respectively. In IM lymphocytes, on the other hand, only the larger band was principally observed, indicating that the p70 is mainly expressed on IM lymphocytes. Furthermore, a band with an apparent mol wt of 55,000 was also visualized in two of three IM patients.

**DISCUSSION**

Earlier studies demonstrated that the lymphocytosis manifested in acute IM is ascribed to the expansion of T lymphocytes, the majority of which are of CD8+ cytotoxic/suppressor phenotype. This expansion is thought to result from the activation and proliferation of T lymphocytes in vivo caused by EBV+ B lymphocytes. Common occurrence of HLA-DR antigen-bearing T lymphocytes in IM is one of the supportive evidences of such an activation process in vivo. However, T lymphocytes from IM patients that do not express Tac antigen/IL-2 receptor present a striking contrast to lectin- or antigen-stimulated T lymphocytes in vitro that usually express Tac antigen. How these Tac-negative T lymphocytes can expand in vivo is yet to be elucidated. In the present study, we showed that PBMC from six acute IM patients which were of CD3+, CD8+, HLA-DR+, Tac-
Fig 4. Effect of anti-Tac (p55) antibody and 2R-B (p70) antibody on proliferative responses to IL-2 of PBMC from patients 4 (A), 5 (B), and 6 (C). PBMC were cultured with various concentrations of IL-2 (●) for 72 hours, and 3H-TdR uptake during the last 6 hours was measured. Anti-Tac antibody (△), 2R-B antibody (○), 2R-B + Tac antibody (■) or control anti-Lyt-2.1 antibody (*) was added to the culture at 630 nmol/L, and their effects were examined.

phenotype as previously reported expressed a considerable amount of IL-2 binding sites and proliferated in response to IL-2. From the results of flow cytometric analysis using 2R-B antibody and affinity cross-linking study, the IL-2 receptor expressed on IM lymphocytes was demonstrated to be the p70, the second chain of the IL-2 receptor. The extra band of an apparent mol wt of 55,000, which was detected by affinity cross-linking study, could be HLA class I antigen cross-linked with IL-2.

IM lymphocytes expressed a larger amount of the p70 than did normal resting T lymphocytes. This was supported by the data of two color stainings using 2R-B antibody, showing that the fluorescence intensity of IM CD8+ lymphocytes was higher than that of normal lymphocytes (data not shown). Recently, Sharon et al. reported that both CD4+ and CD8+ subpopulations of resting T lymphocytes express the p70 in similar fashion to whole PBMC, although the precise quantitative relationship remained unclear. In addition, Bich-Thuy et al. also showed 600 to 700 p70 IL-2 binding sites/cell on resting T cells and that high concentrations of IL-2 produced a delayed (peak at 7 days) but pronounced proliferation of resting T cells. In regard to the augmented expression of the p70 on IM lymphocytes, the following explanations should be considered: (1) the p70 expression was induced on the
levels were elevated in acute IM, although the expanded cells in the peripheral blood did not express Tac antigen. Therefore, we cannot exclude the possibility that T lymphocyte activation involving Tac expression may occur somewhere in the bodies of the IM patients. It can be speculated that T lymphocytes that have proliferated through high affinity IL-2 receptor-mediated paracrine or autocrine mechanism may soon lose Tac antigen and appear in the peripheral blood during acute phase of IM.

With regard to the receptor mainly involved in IL-2–induced cell proliferation in IM, 2R-B antibody completely inhibited the proliferative responses whereas anti-Tac antibody showed a variable inhibitory effect in cases 4, 5, and 6, in which the MoAbs’ inhibitory effects were examined in detail. We cannot exclude the possibility that IM lymphocytes expressing a considerable number of p70 and a small number of p55 (Tac), undetectable by flow cytometric analysis, proliferated in response to IL-2. However, we consider it more likely that p70 IL-2 receptor is mainly involved in IL-2–induced cell proliferation because 2R-B antibody, but not anti-Tac antibody, completely inhibited IL-2–dependent cell proliferation in case 6, in which the proportion of CD8⁺, HLA-DR⁺, 2R-B⁺ lymphocytes was markedly high. A small number of normal (not atypical in IM) lymphocytes contained in isolated PBMC that express both p55(Tac) and p70 of IL-2 receptor, or only p70, and can be induced to express p55 during the culture with IL-2 may be at least partially responsible for observed proliferative responses in cases 4 and 5, in which anti-Tac antibody showed a slight or moderate inhibitory effect. Because the IL-2 receptor (p70) expressed on fresh IM lymphocytes is a functional receptor, it seems likely that it is involved in the immune responses generated by primary EBV infection. Further analysis of IM lymphocytes from various aspects may give some clues to understanding the process in which T lymphocytes are activated without expressing Tac antigen, how they proliferate in vivo, and what is the meaning of such an activated state.

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