A diminished gamma-interferon (IFN-gamma) production by T cells from patients with rheumatoid arthritis (RA) in response to autologous stimulation coincides with a defective regulation of Epstein-Barr virus (EBV) transformation and is in part due to monocyte-produced interleukin-1 (IL-1) inhibitor and prostaglandins. Since IL-2 can act directly on unstimulated T-cells to induce IFN-gamma production we have now examined the effect of recombinant IL-2 (rIL-2) on purified resting T lymphocytes from RA patients. Treatment with rIL-2 (25 U/ml) of lymphocytes from 15 controls led to an increased production of 72-h supernatant EBV-inhibitory activity (19±4% SE without; 48±7% with rIL-2), but had only minimal or no effect on gamma-interferon production by E-rosetting lymphocytes from 15 patients with active RA. This defect could not be corrected by adding indomethacin to RA cultures.

KEY WORDS: Rheumatoid arthritis, Interleukin-2, Gamma interferon, Epstein–Barr virus.

RHEUMATOID arthritis (RA) is associated with subtle immunoregulatory defects that can be in part recognized in the deficient autologous mixed leucocyte reaction (AMLR) [1–3] and in the in vitro and in vivo abnormalities relating to Epstein–Barr virus (EBV) infection [3–10]. We have previously presented evidence that a diminished gamma-interferon (IFN-gamma) production by RA T lymphocytes coincides with a defect of the suppression of the EBV-induced DNA-synthesis [3]. The suppression of EBV-induced B cell proliferation by AMLR-supernatants can be blocked with monoclonal antibody against IFN-gamma which indicates that this assay can be used for detecting small amounts of IFN-gamma [3]. The impaired IFN-gamma production is observed when the RA T cells interact with autologous non-T lymphocytes and may be due in part to prostaglandin and IL-1 inhibitor produced by the adherent cell population [8, 9]. In contrast, when activated in the deficient autologous mixed leucocyte reaction (AMLR) stimulator cells, the RA T lymphocytes can then produce IFN-gamma in normal amounts [8, 9]. AMLR proliferative response and IFN-gamma generation are significantly associated with the production of IL-2 [1, 9, 11–13], which may itself be modulated by monocyte-mediated events [9, 14, 15]. IL-2 can also act directly on unstimulated T cells to induce IFN-gamma production without induction of a mitogenic response [12]. In different assay systems, RA lymphocytes produced less IL-2 than normals and responded poorly to exogenous IL-2 [1, 16, 17]. This study was undertaken to examine the effect of rIL-2 on RA T lymphocytes in the absence of mitogens or AMLR stimulator cells.

MATERIALS AND METHODS

Subjects

Heparinized venous blood was obtained from 15 patients with definite or classical active RA, aged 39–75 (mean 58) years [18]. The control population consisted of 12 normal blood-donors aged 27–76 (mean 45) years. Three patients with active seronegative spondarthropathies all taking nonsteroidal anti-inflammatory drugs (NSAIDs) (two with psoriasis and polyarticular arthritis, one with ankylosing spondylitis, aged 44, 76 and 18 years) were added to the healthy control group. All of the RA patients were taking NSAIDs and, in addition, 11 of the RA patients were receiving either chloroquine, gold or d-penicillamine. Two RA patients were also being treated with small doses (<10 mg/d) of prednisone.

Cell separation

Peripheral-blood mononuclear cells (PBM) were obtained from heparinized venous blood by Ficoll-Hypaque density-gradient centrifugation, and E-rosetting lymphocytes were separated from non-T cells by erythrocyte-rosetting with 2-aminoethylisothiouronium hydro-
bromide (AET, Sigma, St. Louis, MO) treated sheep red blood cells [19]. PBM were depleted of adherent cells by passage through G-10 Sephadex columns [20] in RPMI 1640 supplemented with 10% heat-inactivated FCS. The resultant E+ cell population contained >90% E-rosetting cells and less than 2% esterase positive cells.

**Peripheral blood mononuclear cell proliferative responses**

Adherent cell-depleted lymphocytes were cultured in 0.2 ml RPMI 1640-10% FCS supplemented with 2 mM glutamine and gentamycin (10 µg/ml) in 96-well flat-bottomed Linbro plates (Flow Laboratories, Hamden, CT) in the presence or absence of recombinant IL-2 (gift from Biogen S.A., Geneva). Cells were pulsed with (3H) thymidine (1 µCi/well; sp.act. 5 Ci/mmol, Amersham Corp.) for the last 18 h of incubation. The cultures were harvested on day 3 with a semi-automated multiple-sample harvester (MASH) device. Data are expressed in cpm ± SEM (n = 4).

**Preparation of supernatants**

T cell derived supernatants were produced in the presence and absence of rIL-2 (25 U/ml) in 2 ml RPMI-10% FCS at a cell density of 10⁶ cells/ml. After 72 h, the supernatants were recovered and filtered through 0.45 µm membranes (Millipore Corp., Bedford, MA). The supernatants were stored at −70°C until used.

**EBV and interferon assay**

Transforming virus was obtained from the supernatants of the EBV-secreting B 95-8 marmoset lymphoblastoid cell line. Double-rosetted normal non-T cells were infected with EBV, and the effect of 72-h culture supernatants on EBV-induced DNA synthesis was assessed by measuring (3H) thymidine incorporation on day 10 as described [3]. The data obtained are expressed as percentage suppression calculated as:

\[
\text{Percentage suppression} = \left[ 1 - \frac{\text{cpm in supernatant-treated cultures}}{\text{cpm in control cultures}} \right] \times 100.
\]

Gamma-interferon was measured using a radioimmunoassay (Centocor, Malvern, PA) [21].

**Prostaglandin and IL-2 assay**

Culture fluids were assayed for PGE₂ and for IL-2 as previously described [22].

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Monoclonal anti-Tac antibody and control ascites was provided by T. A. Waldmann, NIH [23].

Student’s t test was used to determine the significance of differences between groups.

**RESULTS**

In the presence of rIL-2, unstimulated E-rosetting lymphocytes from 15 controls released factor(s) into the culture medium which suppressed EBV-induced DNA synthesis as an index of IFN-gamma production (Fig. 1). EBV-induced B cell proliferation, measured 10 days after virus infection, was inhibited by 72-h cul-

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**Fig. 1.**—The effect of rIL-2 on the production of a supernatant inhibitory factor by E-rosetting lymphocytes. 2 × 10⁶ T cells from 12 normal controls (O), three patients with seronegative spondarthropathies (■) and 15 RA patients were incubated in 0.2 ml RPMI 1640-10% FCS in the presence and absence of rIL-2 (25 U/ml). Supernatants collected at 72 h were tested for their ability to suppress EBV-induced DNA synthesis. The results are expressed as the mean percentage suppression of (3H) thymidine incorporation compared with control cultures at 10 days post EBV infection (mean control counts from six experiments = 67811 cpm). Error bars represent the mean ± SEM for all individuals in the designated groups.
tured supernatants from 19.2%±4.0 (mean ± SE) without rIL-2 to 47.8%±6.5 at an rIL-2 concentration of 25 U/ml (p < 0.001). R IL-2 had no direct inhibitory effect on EBV-induced B lymphoblast transformation. Tested on three occasions at 4-week intervals, the IL-2-induced IFN-gamma response from the same normal donor (E.F.) was similar. Three patients with active seronegative spondarthropathies treated with NSAIDs responded similarly to normal donors. In contrast to the inhibitory activity on EBV-induced DNA synthesis generated by T lymphocytes from controls in response to rIL-2, the E-rosetting lymphocytes from 15 RA patients did not generate this inhibitory activity in response to rIL-2.

To confirm that the EBV inhibitory factor was specifically induced by rIL-2, a monoclonal antibody against IL-2 receptor was added at a final concentration of 1:2000 to the culture of a normal donor. At the two concentrations of rIL-2 tested, Tac antibody abrogated completely the induction of interferon (Fig. 2). Tac antibody and control ascites had no significant direct effect on EBV-induced proliferation.

Since a small quantity of adherent cells (judged by esterase staining) always contaminated our cultures (<2%) and since prostaglandins produced by these adherent cells can decrease IFN-gamma generation, indomethacin (1 μg/ml) was added to the cell cultures. PGE2 concentration in non-indomethacin-treated cultures was <4 ng/ml and indomethacin did not affect interferon activity of supernatants (three of three experiments with RA cells, data not shown).

A difference in subsets of lymphocytes or spontaneous IL-2 release due to separation techniques or disease might contribute to the RA defect. To test this possibility, lymphocytes were examined for surface phenotypes and the corresponding cell-culture supernatants, stimulated or unstimulated with rIL-2, were examined for EBV-suppressive activity. In two of two experiments, both rIL-2 responding controls and non-responding RA patients showed similar ratios of OKT3, OKT4 and OKT8 subpopulations (data not shown). IL-2 concentration in unstimulated 72-h T cell supernatants from three control and three RA cultures was <0.01 U/ml.

A difference in supernatant EBV inhibitory activity might be paralleled by a difference in the proliferation of the control and RA lymphocytes exposed to rIL-2. As shown in Table I, (3H) thymidine incorporation on day 3 by purified T lymphocytes was no different in normal and RA cells in the presence or absence of rIL-2.

Using a radio-immunoassay (Centocor IFN-gamma RIA), when the supernatant EBV inhibitory activity was high, gamma-interferon level paralleled the percentage suppression of EBV-induced DNA synthesis (Table II). rIL-2-induced IFN-gamma production by normal cells could be inhibited by the addition of Tac antibody (Fig. 2) and no IFN-gamma was found in non-EBV-inhibitory RA T cell supernatants (Table II).

**DISCUSSION**

We have confirmed that peripheral-blood T lymphocytes from RA patients are deficient in their response to IL-2 [1, 16, 17]. We have shown that unstimulated T cells from normal controls and patients with spondarthropathies in the presence of rIL-2 produce sufficient amounts of gamma-interferon to inhibit in vitro EBV-induced B cell proliferation. By contrast, RA E-rosetting lymphocytes are deficient in their
Results are expressed in cpm ± SEM (n = 3).
* [3H] thymidine incorporation was assessed on day 3 after an 18 h pulse.
† rIL-2 concentration was 25 U/ml.
‡ PHA concentration was 1 µg/ml.

ability to respond to exogenous IL-2. Our data clearly demonstrated that purified rIL-2 is not mitogenic [12], and the difference in IFN-gamma production observed between normal and RA lymphocytes can therefore not be explained by a difference in proliferation. Recently, we have shown that diminution of IFN-gamma generation by RA cells was not due to the effects of either chronic inflammatory disease or drug therapy [3]. The IL-2 and IFN-gamma defect(s) are complex and may involve multiple cell interactions. Pre-activated RA-T cells absorb smaller amounts of IL-2 than control cells suggesting that they have fewer or less-functional IL-2 receptors on their surfaces [16, 17]. Exogenous IL-2 failed to normalize the defective AMLR proliferative response in RA patients [1]. IL-2 regulates the subsequent production of IFN-gamma from T-cells [12] and monocytes are not required for its induction [24]. Kasahara et al. [12] have shown that IL-2 acts directly on unstimulated T cells to induce IFN-gamma production without induction of a mitogenic response. Both natural killer cells and T cells have been shown to produce IFN-gamma in response to IL-2 [12, 13]. Since our assay system was not designed to detect natural killer cells, we cannot exclude the possibility that IFN-gamma was produced by rIL-2 stimulation of these cells. In RA, the production of both lymphokines is down-regulated by monocyte-mediated events [3, 8, 9]. We have previously shown that the diminished EBV-suppressor-factor generation by autologously-activated RA T cells or a T cell subset is related in part to their increased sensitivity to PGE [8]. Lotz et al. have demonstrated that EBV-related T cell defects in RA could be explained on the basis of a monocyte produced IL-1 inhibitor [9]. RA T cells were not detectably defective in their IFN-gamma production when stimulated by allogeneic non-T cells [3] or autologous monocyte-depleted EBV-infected B-cells [9]. Indomethacin treatment of our adherent cell-depleted RA E-rosetting lymphocytes did not correct their diminished responsiveness to exogenous IL-2. Thus, the rIL-2 deficiency of RA-blood T lymphocytes appears to be a distinct cellular defect not directly related to in vitro PGE or IL-1 inhibitor effects. We cannot exclude the possibility that the exposure of lymphocytes to prostaglandins or IL-1 inhibitor in vivo or the presence of inhibitory cells such as in SLE may have affected the RA cell response [25].

Our study has confirmed the deficiency of RA peripheral blood lymphocytes to exogenous IL-2 and has demonstrated an impaired IFN-gamma

<table>
<thead>
<tr>
<th>Table II</th>
<th>Correlation of Percentage Supernatant-mediated Inhibition of EBV-induced B Cell Proliferation with IFN-gamma Level as Assessed by Radioimmunoassay</th>
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<tbody>
<tr>
<td>Donor*</td>
<td>rIL-2†</td>
</tr>
<tr>
<td>RA 1</td>
<td>-</td>
</tr>
<tr>
<td>RA 2</td>
<td>+</td>
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<tr>
<td>Control 1</td>
<td>-</td>
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<td>Control 2</td>
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<td>Control 3</td>
<td>6</td>
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<td>+</td>
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* T cell supernatants were obtained after 72 h of culture.
† 2x10⁶ E-rosetting lymphocytes were cultured with (+) or without (−) 25 U/ml rIL-2.
‡ Results are expressed as the mean of two determinations of percentage suppression of EBV-induced B cell proliferation compared with control cultures at 10 days post EBV infection as described in Materials and Methods.
§ Data are expressed as the mean of two determinations of IFN-gamma as described in Materials and Methods.
production by unstimulated RA T cells in response to rIL-2.

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References


NOTICES

THIRTY-THIRRD ANNUAL MEETING OF THE VIGGO PETERSEN CENTRE: RHEUMATOLOGY UPDATE 1988
President: Prof. S. de Seze
Organizing Committee: Profs. A. Ryckewaert, M. F. Kahn, D. Kuntz, A. Dryll and Dr. Cl. Guerin.
Further information: Melle C. Moroy, Centre Viggo Petersen, 6 Rue Guy Patin, 75010, Paris.

ROYAL NATIONAL HOSPITAL FOR RHEUMATIC DISEASES: 250TH ANNIVERSARY INTERNATIONAL CONFERENCE ON RHEUMATOLOGY
In 1988 the Royal National Hospital for Rheumatic Diseases will be celebrating its 250th anniversary. The major event of the seven-month programme will be an international conference focusing on both historical aspects and state of the art lectures from world-renowned authorities.
For further information contact: Andrei Calin, MD FRCP, Royal National Hospital for Rheumatic Diseases, Upper Borough Walls, Bath BA1 1RL, UK.
Tel.: (0225) 65941.

INTERNATIONAL SOCIETY FOR PROSTHETICS AND ORTHOTICS SCIENTIFIC MEETING
Venue: University of Bath.
Further details: Dr. B. McHugh, ISPO Bath 88, National Centre for Training and Education in Prosthetics and Orthotics, University of Strathclyde, Curran Building, 131 St. James Road, Glasgow G4 0LS, UK.

FOURTH MEDITERRANEAN CONGRESS OF RHEUMATOLOGY FIRST MEDITERRANEAN SYMPOSIUM ON BEHÇET'S DISEASE
Venue: Atatürk Cultural Centre, Istanbul, Turkey.
Main topics: Juvenile chronic arthritis, familial Mediterranean fever, amyloidosis associated with rheumatic diseases, vasculitis in Behçet's disease.
Further information: Prof. N. Dilsen, Istanbul Faculty of Medicine, Division of Rheumatology, Capa, Topkapi, Istanbul, Turkey.
Ridaura

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