All-trans Retinoic Acid Potentiates the Ability of Interferon Beta-1b to Augment Suppressor Cell Function in Multiple Sclerosis

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Objective: To determine the effects of combination all-trans retinoic acid (RA) and interferon beta-1b therapy on immune system functions potentially relevant to multiple sclerosis (MS).

Design: Interferon gamma–secreting cells, T suppressor cell function, and lymphocyte proliferative responses were assayed using peripheral blood mononuclear cells from patients with MS and control subjects under control conditions and in the presence of interferon beta-1b, RA, and the 2 combined.

Setting: A university hospital MS clinic.

Participants: Seventeen patients with secondarily progressive MS and 25 control subjects.

Results: Interferon beta-1b use increased interferon gamma–secreting cell counts, augmented T suppressor cell function, and inhibited T-cell proliferation. Therapy with RA decreased interferon gamma–secreting cell counts, had a minimal positive effect on T suppressor cell function, and had no effect on T-cell proliferation. When RA and interferon beta-1b were combined, the inhibitory effect of RA on interferon gamma–secreting cells predominated, T suppressor cell function increased synergistically over the increment observed with interferon beta-1b use alone, and the inhibitory effect of interferon beta-1b alone on T-cell proliferation remained unchanged.

Conclusions: Treatment with interferon beta-1b partially restores defective T suppressor cell function in patients with MS. This potentially beneficial action is synergistically potentiated by RA. Interferon beta-1b increases the number of interferon gamma–secreting cells in the circulation when treatment is initiated. A similar increment in interferon gamma-secreting cells is observed when interferon beta-1b is added to cultural peripheral blood mononuclear cells in vitro. This potentially deleterious action of interferon beta-1b is reversed by RA. Interferon beta-1b inhibits lymphocyte proliferation modestly but reproducibly. This action of interferon beta-1b is unaltered by RA. These data provide a rationale for a trial of combination treatment with interferon beta-1b and RA in patients with MS.

Interferon beta-1b (Betaseron, Berlex Laboratories Inc, Richmond, Calif) and interferon beta-1a (Avonex, Biogen Inc, Cambridge, Mass) are widely used to treat ambulatory patients with multiple sclerosis (MS). Both preparations reduce MS attack frequency and disease activity as measured by serial magnetic resonance image scanning. Use of interferon beta-1b reduces accumulating disease burden as well. Benefit lasts more than 5 years for interferon beta-1b. Interferon beta preparations are only partly effective; almost all patients treated with these preparations relapse eventually. Perhaps efficacy could be improved if interferon beta were combined with another drug.

Interferon beta-1b inhibits viral replication and cell proliferation and modulates immune responses (reviewed by Arnason and Reder). Any of these actions could bear on its therapeutic efficacy in patients with MS. Viral infections provoke MS attacks, cell proliferation is critical in the generation of immune responses, and aberrant immune system reactivity characterizes MS. Some immune system aberrations correlate with disease activity. Non-specific T suppressor cell function fails with MS attacks, recovers as attacks end, and is persistently subnormal in patients with progressive MS. Interferon beta-1b added to peripheral blood mononuclear cells (PBMCs) from patients with MS increases suppressor cell function, and patients with MS receiving interferon beta-1b exhibit a significant rise in suppressor cell function above pretreatment values. This rise may contribute to benefit.

Interferon gamma administration causes MS attacks. Interferon gamma in the blood is increased during MS attacks, and numbers of circulating interferon gamma–secreting cells also rise. When interferon beta-1b treatment is begun, the number of circulating interferon...
PATIENTS AND METHODS

PATIENTS

Seventeen patients with clinically definite, secondarily progressive MS and 25 healthy control subjects were studied. No patient received corticosteroid or other immunosuppressive treatment during the previous year or interferon beta at any time. Four patients with relapsing-remitting MS taking interferon beta-1b were also studied. Venous blood samples were taken after informed consent was obtained.

Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells were isolated on a Ficoll-Paque gradient (Pharmacia Biotech Inc, Piscataway, NJ) and either studied directly or processed for subset isolation.

Lymphocyte Subset Purification

Monoclonal antibodies used for cell subset purification include OKT8 (anti-CD8), OKT4 (anti-CD4), and OKT3 (anti-CD3) (Ortho Diagnostic Systems Inc, Raritan, NJ); B19 (anti-CD19) (Silenus Laboratories, Hawthorn, Victoria, Australia); MEM 18 (anti-CD14); and NK1-nbl-1 (anti-CD56) (Caltag Laboratories, South San Francisco, Calif). The PBMCs were incubated in 10 mL of tissue culture medium–treated Petri dishes to remove adherent cells. Nonadherent cells were collected, pelleted, and labeled for 45 minutes on ice with 2 to 10 µg of monoclonal murine antibody as follows: for CD4 lymphocyte isolation: anti-CD8, CD56, CD19, and CD14; for CD8 lymphocyte isolation: CD56, CD19, CD14, and CD4; and for CD56 lymphocyte (NK cell) isolation: CD4, CD8, CD14, and CD3.

Cells were then placed in Petri dishes, coated with goat antimouse IgG antibody (Organon Teknika Corp, Durham, NC) to remove T and NK cells and with goat antihuman IgA+G+D+E+M antibody (Organon Teknika Corp) to remove B cells, and kept at room temperature for 2 hours. Nonadherent cells were gently washed off and then centrifuged, and the cell pellets were resuspended and relabeled with antibodies (see the previous paragraph) for 1 hour on ice. The cells were then incubated with rabbit complement (Accurate Chemical and Scientific Corp, Westbury, NY) at a 1:4 dilution in RPMI 1640 medium (Sigma Chemical Co, St Louis, Mo) for 45 minutes at a 37°C water bath. Cells were then overlayed on a Ficoll-Paque gradient and centrifuged at 2000 rpm. Viable cells, isolated from the interface, were washed twice in Hanks balanced salt solution, resuspended, counted, and plated in triplicate wells to which conA (5 µg/mL) was added. Interferon beta-1b (final concentration, 1000 U/mL) was added to some wells. Interferon gamma–positive and lymphotoxin (LT)–positive immunospots were counted as described below.

Monocyte Purification

Peripheral blood mononuclear cells were suspended and labeled with 2 to 10 µg of anti-CD3, CD8, CD19, and CD56 for 1 hour on ice. Cells were then washed with Hanks balanced salt solution and resuspended in rabbit complement (Accurate Chemical and Scientific Corp) at a 1:4 dilution in RPMI 1640 medium and incubated at 37°C in a water bath for 45 minutes. The cells were then resuspended, counted, and plated in triplicate in the presence or absence of 1000-U/mL interferon beta-1b. Interleukin 10 (IL-10)–positive immunospots were counted as described below.

IMMUNOSPOTS

Preparation of Interferon Gamma, IL-10, and LT Immunospot Plates

Monoclonal murine antibodies to human interferon gamma (BioSource International, Camarillo, Tex), IL-10 (Pharmingen, San Diego, Calif), and LT (R&D Systems, Berkeley, Calif) were diluted in carbonate-carbonate buffer to concentrations of 15, 8, and 10 µg/mL, respectively; 50 µL of each was added to separate wells in 96-well nitrocellulose-bottom plates (Millipore Corp, Marlborough, Mass). Plates were then incubated overnight at 37°C; 100 µL of 2% bovine serum albumin was then added to each well for 4 hours at 37°C. Wells were washed with sterile phosphate-buffered saline before use.

Effect of Interferon Beta-1b and RA Treatment on Interferon Gamma–Secreting Cell Counts

One hundred thousand PBMCs in RPMI medium with 5% autologous plasma (final volume, 100 µL) were added to each well of the immunospot plate. Each operation was performed in 3 wells. Cells were incubated with 5-µg/mL conA plus 100- or 10-U/mL interferon beta-1b. All-trans retinoic acid (Sigma Chemical Co) was reconstituted in dimethyl sulfoxide (DMSO) (see below) and then diluted to the desired concentration in medium. All-trans retinoic acid was added to a final concentration of 5 × 10−7 mol/L or 5 × 10−8 mol/L. Wells were also prepared with similarly diluted DMSO to serve as a control. The plates were then covered with tinfoil and incubated for 48 hours at 37°C.

Enumeration of Interferon Gamma, IL-10, and LT Immunospots

After incubation, the cells were discarded and the plates were washed in phosphate-buffered saline. Anti-interferon gamma antibody (Pharminen), biotinylated antihuman IL-10 antibody (Endogen Inc, Boston, Mass), and anti-LT antibody (Boehringer Ingelheim Bioproducts, Ingelheim, San Diego, Calif), and LT (R&D Systems, Berkeley, Calif) were diluted in carbonate-carbonate buffer to concentrations of 15, 8, and 10 µg/mL, respectively; 50 µL of each was added to separate wells in 96-well nitrocellulose-bottom plates (Millipore Corp, Marlborough, Mass). Plates were then incubated overnight at 37°C; 100 µL of 2% bovine serum albumin was then added to each well for 4 hours at 37°C. Wells were washed with sterile phosphate-buffered saline before use.

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Interferon gamma–secreting cells increases during the first 2 months of treatment.37 Interferon beta-1b fails to reduce MS attack frequency during the first month of treatment (J. Wallenberg, MD, oral communication, 1994).

Desired properties in an agent to be considered as the best available animal model for MS.

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Vienna, Austria) were diluted in 0.2% bovine serum albumin in phosphate-buffered saline to a concentration of 8, 10, and 8 µg/mL, respectively; 50 µL of antibody solution was added to the corresponding well and then incubated at 4°C overnight. Plates were again washed with phosphate-buffered saline, and 50 µL of horseradish peroxidase conjugated antibiotin goat antibody for IL-10 (Vector Laboratories Inc, Burlingame, Calif), antimouse IgG1k antibody for interferon gamma, or antimouse IgG2b goat antibody for LT (Caltag Laboratories) was then added to each well for 2 hours at 4°C. Plates were again washed with phosphate-buffered saline, and 100 µL of substrate solution was added to each well. Substrate solution was made by dissolving 350 µg of 3-amino-9-ethyl-carbazole in 2.5 mL of dimethylformamide; 47.5 mL of 0.05-mol/L sodium acetate buffer was added, followed by filtration. Thirty-five microliters of 30% hydrogen peroxide was added to the solution. Plates were stained for 20 minutes to 1 hour at room temperature and then washed with water. Spots, considered cytokine-secreting cells, were enumerated twice using a dissecting microscope (all wells were counted by A.D.). The total number of spots in control wells (always <5) was subtracted from the total number of spots in experimental wells. Control wells were coated with irrelevant isotype-matched monoclonal murine antibody (Caltag Laboratories).

Effect of RA on conA-Induced Proliferation

Peripheral blood mononuclear cells were resuspended at 10⁶ cells/mL in RPMI medium supplemented with 10% fetal bovine serum, 2-mmol/L levoglutamine, 100-U/mL penicillin, and 0.1-mg/mL streptomycin sulfate; 20 µL of conA stock solution (50 µg/mL) in RPMI medium (supplemented as stated above) was added to all microwells. All-trans retinoic acid was dissolved in pure DMSO (Fisher Scientific, Fair Lawn, NJ) at 5 × 10⁻² mol/L and stored at −20°C. The RA and DMSO stock (or DMSO alone as a control) was diluted in RPMI medium and sonicated immediately before use; 20 µL of diluted RA and DMSO or diluted DMSO was added to some wells; 60 µL of RPMI medium was added to wells containing conA plus RA and DMSO or conA plus DMSO followed by 100 µL of cell suspension (10⁵ cells). The final volume was 200 µL per well; the conA concentration was 5 µg/mL; the RA concentration was 5 × 10⁻¹, 5 × 10⁻², or 5 × 10⁻⁵ mol/L; and the DMSO dilution was 10⁻⁴, 10⁻³, or 10⁻⁵ vol/vol. Cells were cultured for 48 hours; 1 µCi per well of tritiated thymidine, 185 gigabecquerel/mmol (Amersham Corp, Arlington Heights, Ill), was added during the last 5 hours of incubation. Incorporated radioactivity in cultures was measured by liquid scintillation spectroscopy.

Effect of RA on Interferon Beta-1b–Induced Inhibition of Cell Proliferation

One hundred thousand PBMCs (100 µL) were added to microwells containing conA (5 µg/mL) plus RA and DMSO or conA plus DMSO (see above). Interferon beta-1b was added to some wells for a final concentration of either 100 or 1000 U/mL. Final volume in all wells was 200 µL. Cells were cultured for 72 hours. Cultures were performed in quadruplicate and 1 µCi per well of tritiated thymidine was added for the last 5 hours of incubation with counting as described above.

Suppressor Cell Assay

In 25-cm² tissue culture flasks, 6 × 10⁶ PBMCs in 6 mL of RPMI medium (see above) were cultured with or without conA (5 µg/mL). All-trans retinoic acid and DMSO, DMSO, or the combination of interferon beta-1b with RA and DMSO were added to selected flasks. After 48 hours, cells were harvested, treated with 100-µg/mL mitomycin (Sigma Chemical Co) for 45 to 60 minutes, washed 3 times in Hanks balanced salt solution, and resuspended at 10⁶ cells/mL in RPMI medium. One hundred microliters of conA-activated mitomycin-treated suppressor cells (5 cells) or nonactivated mitomycin-treated control cells (C cells) was added to U-bottom microwells (Costar Corp, Cambridge, Mass) followed by 100 µL of responder PBMCs (10⁶/mL) freshly isolated from a healthy donor (R cells) plus conA for a final concentration of 5 µg/mL. Four cultures were incubated for 72 hours. Wells were pulsed with 1 µCi of tritiated thymidine for the last 5 hours of incubation.

Percentage of suppression was calculated as follows: 1–[(counts per minute (R+S+conA)/counts per minute (R+C+conA))] × 100.

STATISTICAL ANALYSES

The Student paired 2-tailed t test was used throughout. z Tests were performed to determine whether interferon beta-1b and RA synergize in the induction of conA-activated suppressor function. For these calculations, the change in percentage of suppression induced by interferon beta-1b, RA, or both compared with conA alone was determined. A theoretical additive effect was obtained by summing the changes induced by interferon beta-1b alone and RA alone. Additive values were then compared with actual changes observed when interferon beta-1b and RA were combined, using the z test for 2 sample means.

STUDY OF PATIENTS RECEIVING INTERFERON BETA-1b

Blood samples were collected immediately before starting interferon beta-1b treatment and 1 week later. Peripheral blood mononuclear cells were studied for LT-secreting cells as described previously here.
symptoms rather than the “flu-like” symptoms seen with interferon beta-1b use; (5) the increased interferon gamma secretion observed in vitamin A–deficient mice is reversed by retinoid treatment, pointing to an inhibitory RA effect on interferon gamma–secreting cells; (6) retinoids augment nonspecific suppressor cell function; (7) retinoids protect against EAE.

We show that RA, at concentrations achieved in vivo by current treatment regimens, synergistically potentiates the ability of interferon beta-1b to augment suppressor cell function in vitro. Therapy with RA alone exerts trivial effects on human suppressor cell function and does not alter concanavalin A (conA)–driven proliferation of PBMCs. Treatment with RA inhibits generation of interferon gamma–secreting cells and reverses the ability of interferon beta-1b to do so. These properties provide a rationale for a clinical trial of combination treatment with interferon beta-1b and RA in patients with MS.

**RESULTS**

**CYTOKINE-SECRETING CELLS**

Interferon Beta-1b Induces Interferon Gamma Secretion by CD4, CD8, and NK Cells

Control subjects were studied. The number of interferon gamma–secreting cells is increased over control values for CD4, CD8, and NK cells exposed to interferon beta-1b (Table 1). The interferon beta-1b effect is statistically significant for all subsets.

The Interferon Beta-1b–Induced Increase in Interferon Gamma-Secreting Cell Counts Is Transient

Control PBMCs were plated at 0 hours, exposed to conA or conA plus interferon beta-1b, and studied at 24, 36, and 48 hours. Other cell aliquots were incubated for 48 hours in the presence of conA alone or conA plus interferon beta-1b and then plated for an additional 48 hours. Cells plated at 0 hours and counted at 24, 36, and 48 hours showed more interferon gamma-secreting cells when incubated with interferon beta-1b than when incubated with conA alone. Numbers increased over time (Table 2). In contrast, the number of interferon gamma-secreting cells at 96 hours was significantly greater in PBMC cultures treated with conA alone than in the interferon beta-1b–treated group.

RA Inhibits Interferon Gamma-Secreting Cells

Interferon beta-1b (100 U/mL) increases the number of interferon gamma–secreting cells in control PBMC cultures exposed to conA for 48 hours (Figure). The effect is modest but reproducible (paired t test, P < .001) (Figure). All-trans retinoic acid (5 × 10⁻⁹ mol/L) inhibits the generation of interferon gamma-secreting cells (P < .001). When RA and interferon beta-1b are given jointly, the inhibitory RA effect abrogates the stimulatory effect of interferon beta-1b (P < .001). Comparable and equally significant results were obtained with interferon beta-1b at 10 U/mL plus RA at 5 × 10⁻⁷ or 5 × 10⁻⁹ mol/L and with interferon beta-1b at 100 U and RA at 5 × 10⁻⁹ mol/L (data not shown).

**LT-Secreting Cells**

Interferon beta-1b treatment in vitro inhibits the number of LT-secreting cells (Table 3). Secretion of LT is confined to CD4 cells, as expected. Studies ex vivo of PBMCs from patients with MS receiving interferon beta-1b...
reveal a decline in the number of LT-secreting cells in PBMCs isolated 1 week after initiation of interferon beta-1b therapy compared with pretreatment values.

**IL-10–Secreting Cells**

Interferon beta-1b treatment in vitro increases the number of cells that spontaneously secrete IL-10. Monocytes are responsible for the effect (Table 4).

**CELL PROLIFERATION**

Use of RA fails to alter the inhibitory effect of interferon beta-1b on conA-induced PBMC proliferation in vitro. Six control subjects and 4 patients with MS were studied. Results were comparable throughout and similar for both groups. Data for a representative control are given in Table 5. Interferon beta-1b inhibits conA-induced proliferation; 1000 U/mL inhibits more profoundly than 100 U/mL. Dimethyl sulfoxide (diluent for RA) and RA at $5 \times 10^{-7}$ or $5 \times 10^{-8}$ mol/L do not alter the interferon beta-1b effect. All-trans retinoic acid alone at $5 \times 10^{-8}$, and $5 \times 10^{-7}$ mol/L and DMSO at dilutions of $10^{-6}$, $10^{-5}$, and $10^{-4}$ vol/vol do not alter lymphocyte proliferation (data not shown).

**SUPPRESSOR CELL FUNCTION**

When given in combination with interferon beta-1b, RA potentiates nonspecific suppressor function (Table 6). Patients with progressive MS exhibit lower baseline suppressor function than control subjects. Interferon beta-1b therapy compared with pretreatment values. The interferon beta-1b effect is greater at 1000 U/mL than at 100 U/mL. Treatment with RA and interferon beta-1b leads to a significant increase in suppressor function over that achieved with interferon beta-1b use alone. The combination is synergistic (z test for synergy: $P = .02$ for A, $P = .14$ for B, $P = .02$ for C, and $P = .03$ for D in Table 6).

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Table 3. Effect of Interferon Beta-1b on Lymphotoxin-Secreting Cells*

<table>
<thead>
<tr>
<th>Group†</th>
<th>Concanavalin A + Interferon Beta-1b (1000 U/mL)‡</th>
<th>Significance§</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁶ control PBMCs (6)</td>
<td>148.8±11.2</td>
<td>95.3±8.8</td>
</tr>
<tr>
<td>2×10⁶ control CD4 (6)</td>
<td>35.2±3.4</td>
<td>17.7±7.2</td>
</tr>
<tr>
<td>2×10⁶ control CD8 (6)</td>
<td>4.3±1.8</td>
<td>1.9±0.7</td>
</tr>
<tr>
<td>10⁶ MS PBMCs (before treatment) (4)</td>
<td>105±11.9</td>
<td></td>
</tr>
<tr>
<td>10⁶ MS PBMCs (1 wk on interferon beta-1b) (4)</td>
<td>72±7.5</td>
<td></td>
</tr>
</tbody>
</table>

* PBMCs indicates peripheral blood mononuclear cells; MS, multiple sclerosis.
† Mean number of paired observations is shown in parentheses.
‡ Mean number of lymphotoxin–secreting cells±SEM/10⁵ plated cells.
§ Paired 1-tailed Student t test.
||Versus pretreatment values.

Table 4. Effect of Interferon Beta-1b on Interleukin (IL)-10–Secreting Cells

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>Cells Plated*</th>
<th>Interferon Beta-1b (1000 U/mL)</th>
<th>Significance‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10⁶ control PBMCs§ (6)</td>
<td>145.9±36.4</td>
<td>212.0±48.7</td>
</tr>
<tr>
<td></td>
<td>1×10⁶ control monocytes (CD14+) (8)</td>
<td>133.8±39.4</td>
<td>190.5±46.2</td>
</tr>
<tr>
<td></td>
<td>1×10⁶ control CD4 (3)</td>
<td>1.5±1.5</td>
<td>0.3±0.3</td>
</tr>
</tbody>
</table>

* Number of paired observations is shown in parentheses.
† Mean number of IL-10–secreting cells±SEM/number of cells plated.
‡ Paired 1-tailed Student t test.
§ PBMCs indicates peripheral blood mononuclear cells.

Table 5. Lack of Effect of All-trans Retinoic Acid (RA) on T-Cell Proliferation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Concanavalin A*</th>
<th>Concanavalin A + Interferon Beta-1b (1000 U/mL)*</th>
<th>Concanavalin A + Interferon Beta-1b (1000 U/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>45 128±1429</td>
<td>30 670±4158</td>
<td>22 587±2488</td>
</tr>
<tr>
<td>Dimethyl sulfoxide (vehicle)</td>
<td>47 167±966</td>
<td>28 693±3564</td>
<td>21 912±6671</td>
</tr>
<tr>
<td>5×10⁻⁶ mol/L RA</td>
<td>47 587±2818</td>
<td>30 155±4034</td>
<td>25 158±1925</td>
</tr>
<tr>
<td>5×10⁻⁵ mol/L RA</td>
<td>48 424±4662</td>
<td>30 271±2775</td>
<td>24 389±4720</td>
</tr>
</tbody>
</table>

*Mean counts per minute±SEM of 4 cultures from a representative control subject.

Table 6. Potentiation of the Suppressor Cell Function Augmenting Action of Interferon Beta-1b by All-trans Retinoic Acid (RA)*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Multiple Scissors (A)</th>
<th>Control (B)</th>
<th>Multiple Scissors (C)</th>
<th>Control (D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) DMSO†</td>
<td>15.1±2.7 (7)</td>
<td>19.3±5.7 (7)</td>
<td>16.4±3.5 (7)</td>
<td>22.8±5.4 (7)</td>
</tr>
<tr>
<td>(2) RA (5×10⁻⁷ mol/L)</td>
<td>21.8±5.1 (7)</td>
<td>24.3±3.8 (7)</td>
<td>35.0±5.5 (7)</td>
<td>37.0±3.7 (7)</td>
</tr>
<tr>
<td>(3) Interferon beta-1b (1000 U/mL)+DMSO</td>
<td>37.3±3.9 (13)</td>
<td>40.5±3.9 (12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4) Interferon beta-1b (1000 U/mL)+RA (5×10⁻⁷ mol/L)</td>
<td>37.3±3.9 (13)</td>
<td>40.5±3.9 (12)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P values are as follows: A1 vs A2, <.006; A1 vs A4, <.001; A2 vs A3, <.003; A2 vs A4, <.003; A3 vs A4, <.002; B1 vs B2, 22; B1 vs B3, <.001; B1 vs B4, <.001; B2 vs B3, <.001; B2 vs B4, <.002; C1 vs C2, 4; C1 vs C3, 2; C1 vs C4, <.006; C2 vs C3, 26; C2 vs C4, <.006; C3 vs C4, <.01; D1 vs D2, 25; D1 vs D3, 18; D1 vs D4, <.003; D2 vs D3, 74; D2 vs D4, <.003; D3 vs D4, <.006.
†DMSO indicates dimethyl sulfoxide.
At a concentration attained in the blood with current therapeutic regimens, RA synergistically potentiates the capacity of interferon beta-1b to augment nonspecific suppressor cell function in vitro for patients with secondarily progressive MS and healthy control subjects. Nonspecific suppressor cell function is markedly reduced during MS attacks, rebounds with recovery, and is persistently subnormal in patients with progressive MS, as shown here and earlier. Nonspecific suppressor cell function is mediated largely by CD8 cells. CD8 knockout mice lack CD8 cells and, in mice of strains in which EAE is ordinarily a monophasic illness, CD8 knockouts relapse. The finding points to a role for CD8 cells in prevention of EAE relapses and to a possible role for them in the prevention of MS relapses. Patients with MS receiving interferon beta-1b exhibit a significant rise in nonspecific suppressor cell function over baseline values obtained immediately before the start of treatment. This action of interferon beta-1b may bear on its ability to lessen MS attack frequency. For this reason, RA merits consideration as an adjunct to interferon beta-1b treatment.

Interferon beta-1b exerts an antiproliferative effect. Clinically, this translates into a mild lymphopenia and sometimes a mild anemia. If RA is to be considered for combination therapy with interferon beta-1b, it becomes important to determine whether it potentiates the antiproliferative action of interferon beta-1b. We show that RA itself does not inhibit conA-driven T-cell proliferation in vitro, and it does not potentiate the ability of interferon beta-1b to do so. Thus, the ability of RA to potentiate T suppressor cell function cannot be explained by an effect on proliferation. We are aware that at concentrations of conA higher or lower than the 5 µg/mL used by us, RA inhibits T cell proliferation. In addition, RA potentiates the ability of class I interferons to inhibit growth of certain tumor cell lines. We undertook a phase 1 (safety) trial with the retinoid etretinate (Tegison, Hoffmann-LaRoche Inc, Nutley, NJ) as adjunctive retinoid therapy in patients with MS receiving interferon beta-1b. No alteration in blood cell counts beyond those seen with interferon beta-1b therapy alone has been observed.

When interferon beta-1b treatment is begun, there is an early surge in the number of circulating interferon gamma–secreting cells. Flulike symptoms are frequent at this time, presumably because interferon gamma induces production, by monocytes and macrophages, of the endogenous pyrogens tumor necrosis factor and IL-1b. Flulike symptoms usually abate over the first month or 2 of treatment, and the interferon gamma–secreting cell surge wanes as well. No decline in MS attack frequency is observed during the first month of interferon beta-1b treatment. Interferon gamma administration provokes MS attacks so that the interferon gamma–secreting cell surge noted at the onset of interferon beta-1b treatment could pose a risk. Since attack frequency neither increases nor decreases early during interferon beta-1b treatment, the possibility that there is some counterpoise to the potentially deleterious interferon gamma effect merits consideration. The number of LT-secreting cells falls promptly when interferon beta-1b treatment is begun, as shown here. Interferon beta-1b added to cultured PBMCs was earlier shown to markedly inhibit LT production. Because LT damages oligodendrocytes, reduced LT production may offset the hazard posed by the rise in interferon gamma–secreting cells.

We show that interferon beta-1b increases interferon gamma–secreting cell numbers in vitro in CD4 cells (putative Th1-type cells), CD8 cells, and NK cells, the 3 types of lymphocytes known to make interferon gamma. We also show that the increase is transient and that by 96 hours cells secreting interferon gamma in control cultures outnumber those in cultures exposed to interferon beta-1b. This finding points to an early on signal for interferon gamma production and a delayed off signal. The off signal may be dependent on, or contributed to by, cytokines that inhibit interferon gamma production and that are induced by interferon beta-1b. Interleukin 10 provides such an inhibitory signal, and we find increased numbers of IL-10-secreting cells in cultures exposed to interferon beta-1b. Others report increased IL-10 secretion from macrophages exposed to interferon beta-1b or interferon beta-1a.

An inhibitor of interferon gamma secretion might be a desirable adjunct when interferon beta therapy is begun. Earlier, Dayal et al showed that prednisone (10 mg 3 times a day) given during the first few weeks of therapy prevents the interferon gamma–secreting cell surge and substantially attenuates systemic adverse effects. We show here that, at least in vitro, RA therapy lowers interferon gamma–secreting cell numbers and reverses the ability of interferon beta-1b to increase interferon beta–secreting cell numbers. The data suggest that RA might be considered as a treatment to prevent the interferon gamma–secreting cell surge. Inasmuch as interferon gamma levels are elevated during MS attacks and suppressor function is attenuated, a yin-yang between the 2 has been proposed. It is possible that the ability of RA to inhibit interferon gamma secretion shown here, and amply documented in animal experiments, may contribute to its augmenting effect on suppressor function. Against this postulate is the finding that added interferon gamma has no effect on the capacity of interferon beta-1b to augment suppressor cell function. A more probable basis for the RA effect lies, in our estimation, in the ability of RA to potentely induce production of downstream elements such as STAT 1, interferon regulatory factor 1, and interferon regulatory factor 2 used in interferon beta signaling pathways.

Retinoids shift the immune repertoire away from Th1-type responses, including interferon gamma release, toward Th2-type cell-mediated responses. This is thought to be the basis for their beneficial effect in EAE. The role of retinoids in CD8 cell function (a major contributor to nonspecific suppression) remains poorly defined, but CD8 cells secrete much the same panoply of cytokines as CD4 cells. In vitro results must be validated in vivo, and it remains to be seen whether retinoids will potentiate the efficacy of interferon beta-1b in patients with MS.
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