Increased CD80^{+} B Cells in Active Multiple Sclerosis and Reversal by Interferon β-1b Therapy

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Abstract

Costimulatory molecules help determine T cell responses. CD80 (B7-1) and CD86 (B7-2), costimulatory proteins on antigen-presenting cells, bind to CD28 on T cells. When costimulation is coupled with a signal through the T cell receptor (TCR), T cell proliferation and cytokine secretion are induced. However, TCR signaling without CD80/CD86-CD28 costimulation causes anergy.

During multiple sclerosis (MS) exacerbations, circulating immune cells are activated, Th1 cytokine levels in the blood are elevated, and blood-derived immune cells destroy brain oligodendroglia. In the experimental autoimmune encephalomyelitis model of MS, CD80 on antigen-presenting cells induces Th1 cell responses; CD86 enhances generation of Th2 cells. Variation in CD80 and CD86 expression is likely to influence immune regulation in MS.

We demonstrate that the number of circulating CD80^{+} lymphocytes is increased significantly during MS exacerbations, but is normal in stable MS. These CD80^{+} lymphocytes are predominantly B cells, based on two-color flow cytometry. The number of CD71^{+} and HLA-DR^{+} lymphocytes and monocytes is also increased in active MS.

Therapy with IFN β-1b markedly reduces the number of circulating CD80^{+} B cells and increases CD86^{+} monocyte number. HLA-DR^{+}, CD71^{+}, and CD25^{+} mononuclear cell numbers are also reduced by therapy. The number of CD80^{+} cells may be a useful surrogate marker during IFN-β therapy, and reduction of CD80-mediated costimulation may be one therapeutic mechanism by which IFN-β acts in MS. (*J. Clin. Invest.* 1997. 99:2664–2671.) Key words: B lymphocyte • CD80 • CD86 • interferon-β • multiple sclerosis

Introduction

Two signals are needed for T cell activation. Engagement of the T cell receptor (TCR)[1] by an Ag-MHC complex provides the first. Second signals are provided by cytokines such as IL-2, and by costimulatory proteins such as CD80 and CD86, expressed on antigen-presenting cells (APC) (monocytes, dendritic cells, and activated B cells). Binding of CD80/CD86 to CD28, their ligand on T cells, is the most important of the known costimulatory interactions. Joint signaling through TCR-peptide-MHC and through CD28-CD80/CD86 causes activation, proliferation (1–3), and up to 50-fold increases in T cell cytokine secretion (4, 5). However, signaling solely through the TCR, absent costimulation, causes T cell anergy (6). Resting B cells express minimal amounts of CD80 or CD86, and anergize T cells. Activated B cells express high levels of CD80 and CD86, and stimulate T cell responses (3, 7).

CD80^{+} cells activate Th1 cells in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS) (8, 9). In this model, Th1 cell activation leads to delayed-type hypersensitivity (DTH) reactions with secretion of IL-2, IFN-γ, and lymphotoxin (TNF-β). CD86 activates Th2 cells, enhancing humoral immunity and secretion of IL-4 and IL-10 (8, 9). Resolution of EAE correlates with the appearance of Th2 cytokines (10–12). IL-4 and IL-10 inhibit Th1 cell activation and are protective in EAE. In addition to modifying the type of T cell responses, CD80 and CD86 molecules affect the tempo of immune responses. CD86 initiates immune reactions, as it is more rapidly induced than CD80 (7). CD80 may be the more important costimulatory molecule during chronic inflammatory conditions (3).

CD80 and CD86 expression regulates EAE severity and is potentially relevant in MS. Actively induced monophasic EAE in mice is prevented by intraperitoneal treatment with anti-CD80 or with CTLA4-Ig fusion protein (8, 13–15). These agents block the Th1 activation required for generation of the antibrain response. Chronic relapsing EAE (CR-EAE) provides a closer approximation of MS than the monophasic form of the disease. Infusion of anti-CD80 Fab fragments prevents epitope spreading and ameliorates relapses of CR-EAE (16). Finally, CD80 expression on activated microglia, infiltrating macrophages (17, 18), and perivenular lymphocytes (18, 19) is prominent in active MS brain lesions but not in normal brain.

We hypothesized that CD80/CD86 molecules, as well as class II MHC proteins and other activation markers (20), would be overexpressed on peripheral APC in active MS. We find that the number of circulating CD80^{+} B cells, and the CD80/CD86 ratio on B cells and monocytes, is increased during active MS. During IFN β-1b therapy, which reduces MS attacks, the number of CD80^{+} B cells in MS becomes subnormal and the CD80/CD86 ratio approximates control levels. These data indicate that CD80 expression links to disease activity.

1. *Abbreviations used in this paper:* APC, antigen-presenting cell; EAE, experimental autoimmune encephalomyelitis; MNC, mononuclear cell; MS, multiple sclerosis; TCR, T cell receptor.

This reduction of CD80/CD86 expression, impeding costimulation of T cells, may be one therapeutic mechanism of IFN-β.

Methods

Subjects. 15 patients had active MS, defined as the onset of a clinical exacerbation within 2 wk of blood drawing ($n = 9$), or significant progression of disease ($n = 6$) defined by a one point increase in the expanded Kurtzke Disability Status Scale (EDSS [21]) score within the prior year. Eight were women and seven men; average age was 44.5±2.7 yr. 16 stable MS patients, with a history of relapsing/remitting disease, had no clinical disease activity for 6 mo. 14 were women and 2 men; average age was 39.7±2.4. EDSS scores ranged from three to seven in both patient groups. Healthy controls consisted of 18 women and 12 men; average age was 39.6±2.0.

12 MS patients (8 active, 4 stable) were studied before (baseline), and within 9 mo after starting subcutaneous injections of IFN β-1b (Betaseron) 8 million U, every other day. IFN β-1b was from Berlex Laboratories, Richmond, CA.

Reagents. mAb used for flow cytometry included PE-conjugated S4.1 (anti-CD3; T cells), PE-conjugated MEM-18 (anti-CD14; monocytes), PE-conjugated SJ25-C1 (anti-CD19; B cells), PE-conjugated NKI (anti-CD56; NK cells), PE-conjugated NE-51 (anti-CD28) (which were obtained from Chromaphobe Inc., Mountain View, CA). HL38 (anti–HLA-DR) was from Caltag Labs, South San Francisco, CA. OKT10 (anti-CD71; transferrin receptor) was from Ortho-mune mAb, Raritan, NJ. 2A3 (anti-CD25; IL-2 receptor) and L307.4 (anti-CD80, anti–B7-1) were from Becton Dickinson Immunocytometry Systems, Mountain View, CA. B1.1 (anti-CD80, anti–B7-1) was from Repligen Corp., Cambridge, MA. BB1 (anti-CD80, anti–B7-1 and anti–B7-3) and IT2.2 (anti-CD86, anti–B7-2) were from PharMingen, San Diego, CA. Purified mouse IgG_1 and IgG_{2b} from Caltag were used as isotype controls; FITC-conjugated goat $F(ab')_2$ anti–mouse $IgG_{H\&L}$ from Caltag Labs was used as a secondary reagent.

Cell separation. Mononuclear cells (MNC) were collected in heparinized vacutainer tubes and separated on Ficoll-Hypaque density gradients. MNC were washed thrice in HBSS and stained for flow cytometry. To determine B7 expression, $3 \cdot 10^6$ MNC/ml were activated for 48 h with 1,000 U/ml IFN β-1b (Berlex Laboratories), in media (RPMI-1640 plus 5% FBS) and then stained.

Flow cytometry. MNC were suspended in staining media (HBSS, 0.1% BSA, 0.01% Na azide) on ice. 10^6 cells in 50 μl of medium were added to wells of a 96-well bottom plate. Cells were stained with first step reagents for 30 min, washed twice in staining media, and then stained with FITC-conjugated goat anti–mouse IgG if needed. This was followed by a third round of staining with PE-conjugated mAb against subset markers. To control for day-to-day variation in staining and flow cytometer settings, MNC obtained by leukocytapheresis from a single donor, were purified on a Ficoll-Hypaque gradient, aliquotted, and stored on liquid N_2. An aliquot of these control cells was thawed and stained with each experiment.

Double staining with directly conjugated BB1-PE (IgM) and L307.4-FITC (IgG) was used to determine whether cells expressed the B7-1 or B7-3 epitopes of CD80. The BB1 mAb is widely used in human studies, but it recognizes both B7-1 and B7-3 epitopes of CD80. B1.1 and L307.4 are specific for B7-1. BB1 was added 30 min before L307.4 and cells were then incubated for 30 min longer. Sequential addition was necessary because the affinity of the two mAbs for CD80 appeared to differ. Analysis of staining kinetics showed that one-half hour preincubation with BB1 was optimal for dual staining.

MNC were gated on lymphocyte or monocyte peaks based on forward light and side scatter for single marker analysis, or were gated on specific-stained populations such as CD3, CD14, CD19, and CD56 for dual marker analysis. 10,000 cells were analyzed in each sample, using a FACScan® from Becton Dickinson Immunocytometry Systems equipped with LYSIS II software. CD80/CD86+ cell subpopulations were determined with two-color flow cytometry of double-stained cells. Gates for positive and negative populations were set in a similar fashion for all groups. Isotype control Abs were used to determine any nonspecific Ab binding.

Statistics. Unpaired Student's *t* tests (22) were used to compare active and stable MS and control groups. Paired *t* tests (22) were used to compare pre- vs. post–IFN β-1b values. Values are given as mean±SEM. CD80/CD86 ratios are based on the average of individual CD80/CD86 pairs, and not on the average of all CD80 vs. the average of all CD86.

Results

CD80 expression on MS lymphocytes correlates with disease activity. Lymphocytes expressing CD80 were increased three-fold in active MS compared with both stable MS ($P < 0.003$) and normal controls ($P < 0.0001$) (Fig. 1 *A*). There was no difference in CD80 expression between relapsing/remitting patients in exacerbation, versus patients with active secondary progressive MS (data not shown). CD86+ lymphocyte number also tended to be higher in active MS than in stable MS, though not significantly ($P < 0.11$). The number of CD86+
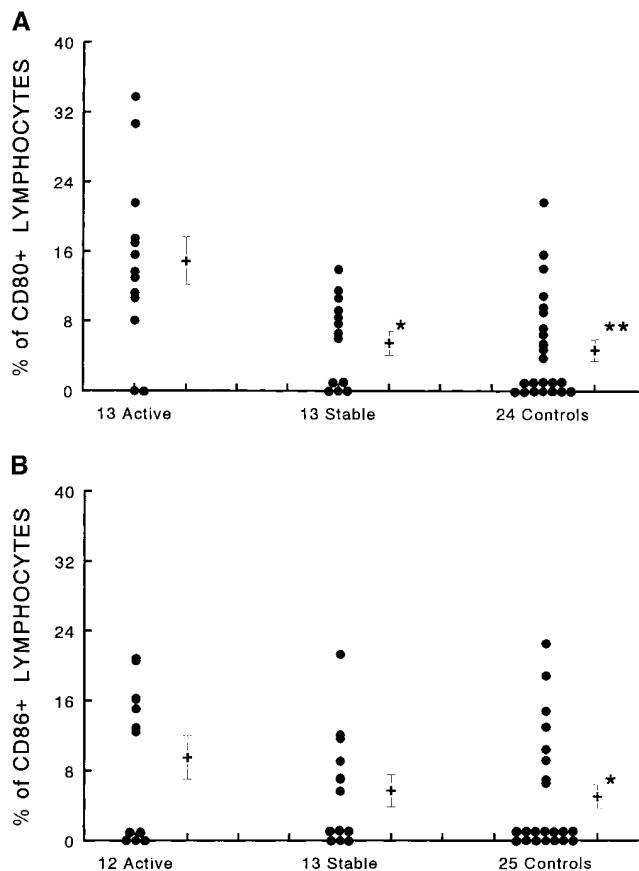
Figure 1. The average percentage of CD80+ and CD86+ lymphocytes in active and stable MS and in healthy controls. (*A*) CD80+ (B7-1+ using B1.1 mAb) lymphocytes were increased in patients with active MS ($n = 13$) compared with stable MS ($n = 13$) (*$P < 0.003$; one-tailed, unpaired Student's *t* test) and controls ($n = 24$) (**$P < 0.0001$). (*B*) CD86+ lymphocytes are more frequent in active MS ($n = 12$) than controls ($n = 21$) (*$P < 0.02$). The decrease in the number of CD86+ lymphocytes in active MS compared with stable MS ($n = 13$) was not statistically significant ($P < 0.11$). Lymphocytes were selected by forward light scatter and side scatter. Error bars are ±SEM.
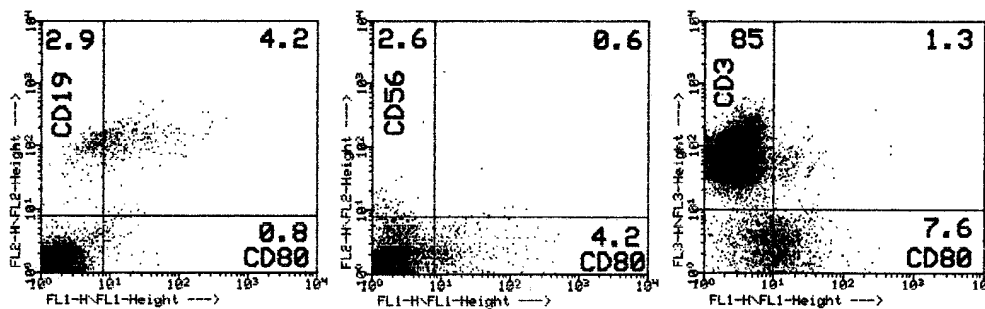
Figure 2. Representative dot plots of lymphocytes stained for CD80 versus CD19 (B cells), CD56 (NK cells), and CD3 (T cells). Data are representative peripheral blood profiles from one of seven MS patients.

cells was elevated in active MS compared with controls ($P <$ 0.02), but to a lesser extent than CD80$^+$ cells (Fig. 1 *B*).

The CD80$^+$/CD86$^+$ lymphocyte ratio was greater in active MS (4.2±1.8) than in stable MS (1.9±1.2), and was threefold higher than the control ratio (1.4±0.3). Thus, expression of CD80/CD86 proteins correlates with disease activity in MS.

Two-color flow cytometry of lymphocytes showed that the CD80$^+$ cells were largely B cells, in both MS and normal subjects. 68±8% of B cells were CD80$^+$ in MS (Fig. 2). In contrast, only 3.7±2.7% of T cells and 17.9±18% of NK cells were CD80$^+$ (Fig. 2). CD86$^+$ lymphocytes were also largely B cells; 57±8% of B cells, but only 5.5±4.7% of T cells and 13.4±13% of NK cells were CD86$^+$ in MS. When present in controls, CD80$^+$ and CD86$^+$ lymphocytes were also predominantly B cells (data not shown). MNC from both MS patients and controls contain $\sim$ 75% T cells, 10% B cells, 5% NK cells, and 10% monocytes. In active MS it follows that up to 60% of circulating CD80$^+$ and CD86$^+$ lymphocytes are B cells. Thus, there is a large reservoir of B cells in active MS that expresses CD80/CD86 costimulatory molecules.

CD80 and CD86 expression on MS monocytes. The frequency of CD80$^+$ monocytes tended to be higher in active compared with stable MS (Fig. 3 *A*). The number of CD86$^+$ monocytes in active MS was not significantly different from stable MS or control values (Fig. 3 *B*). The CD80/CD86 ratio also tended to be greater in monocytes in active MS (3.6±2.8) than in stable MS (2.9±2.0) or controls (2.0±1.3).

Thus, during active disease, CD80 increases more than CD86 on B cells and monocytes. This suggests a role for CD80 costimulatory function in the activation or perpetuation of inflammation in MS.

CD80 recognition by mAb directed against the B7-1 epitope compared with mAb against B7-1 and B7-3 epitopes. Both BB1 (B7-1 and B7-3) and L307.4 (B7-1 specific, equivalent to mAb B1.1 used in Figs. 1–3, 5, and 6) stained lymphocyte subpopulations (Fig. 4, *A–C*). In dual fluorescence staining, a subset of BB1$^+$ lymphocytes did not stain with L304.7 (Fig. 4 *D*). In contrast, the BB1 mAb caused a shift of L307.4$^+$ cells to the right. This indicates that BB1 stains an additional epitope (B7-3) of the CD80 molecule.

In separate experiments, lymphocytes from three controls and five active and three stable MS patients, with disease profiles similar to the patients in Figs. 1 and 3, also showed increased expression of the antigen recognized by L307.4 (B7-1) supporting the data in Figs. 1 and 3. Lymphocyte BB1 (B7-1 plus B7-3) expression was also increased. (Active MS = 1.7±0.8%; stable MS = 0.23±0.04%; NL = 0.85±0.82%; active vs. stable MS, $P < 0.05$).

These data support the demonstration of an increase in B7-1 staining in Figs. 1 and 2, and suggest that the BB1 mAb also detects the increase.

Expression of HLA-DR and CD71 correlates with disease activity. APC express class II MHC proteins, in addition to costimulatory molecules. Class II MHC molecules are required for the primary signal in helper T cell activation. Levels of MHC class II proteins are also an indicator of cell activation. HLA-DR is likely to synergize with CD80/CD86, leading to a more vigorous immune response (23). We therefore measured HLA-DR expression. The numbers of HLA-DR$^+$ pe-
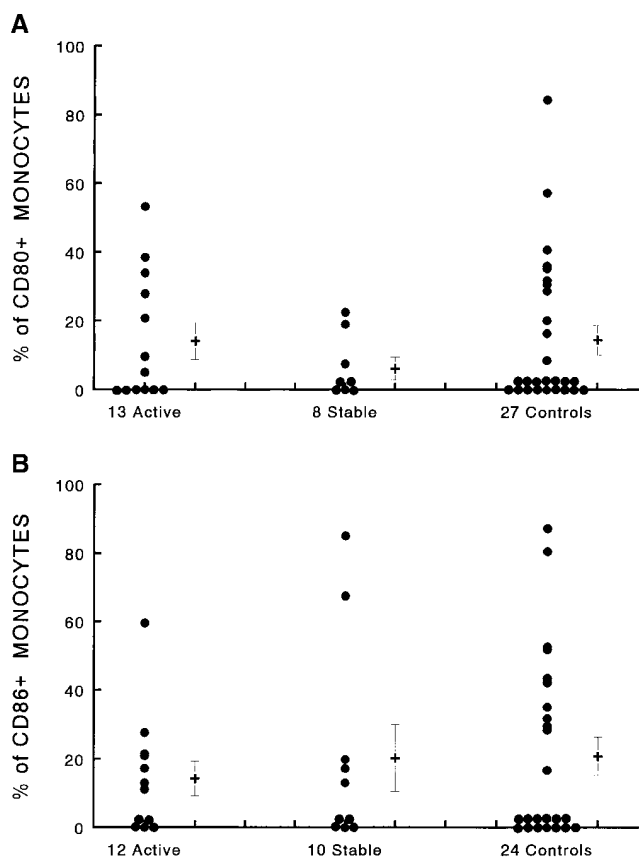

Figure 3. CD80$^+$ and CD86$^+$ expression on monocytes in active and stable MS and in healthy controls. (*A*) The percentage of CD80$^+$ monocytes tended to be greater in active MS than in stable MS ($P <$ 0.06), and lower in stable MS than in controls, ($P < 0.09$) (B1.1 mAb). (*B*) The number of CD86$^+$ monocytes in active MS did not differ significantly from stable MS or controls. Monocytes were separated from other MNC by forward light scatter and side scatter. Error bars are ±SEM.
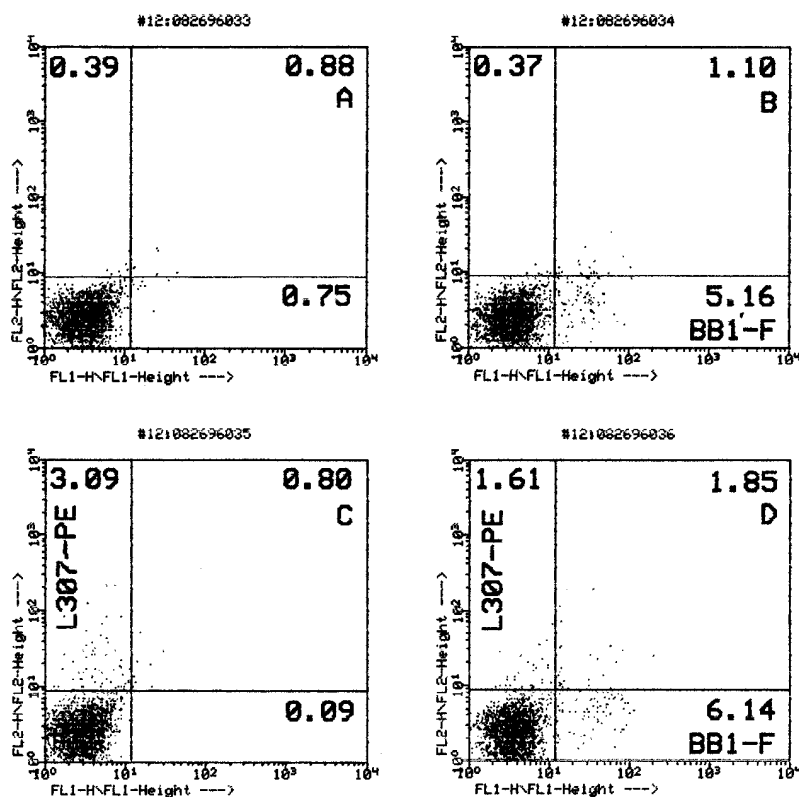
Figure 4. Partial overlap in CD80 staining between L307.4 and BB1 mAbs. MNC were activated with 1,000 U/ml IFN β-1b for 48 h. FACS was gated on lymphocytes. (*A*) Unstained. (*B*) BB1-FITC alone. (*C*) L307.4-PE alone. (*D*) BB1-FITC followed by L307.4-PE causes a shift in L307.4-PE staining to the right, but a subpopulation of BB1-FITC$^+$ cells are not shifted upwards.

ripheral blood lymphocytes and monocytes were significantly increased in active disease, compared with stable MS and controls (Table I). This increase reflects peripheral immune activation.

Transferrin receptor (CD71) expression increases after immunocyte activation. CD71$^+$ lymphocytes and monocytes were two- to threefold more frequent in active MS compared with controls (Table I). In stable disease, CD71$^+$ lymphocytes were 40–50% lower than control levels, indicating a link between inactivity of clinical disease and quiescent immune responses. In stable MS, this overshoot beyond normal levels is also seen in suppressor T cell function (24) and in CD8 expression on T cells (25).

IFN β-1b therapy downregulates lymphocyte CD80 expression. IFN β-1b therapy reduces the number of exacerbations in relapsing–remitting MS. We postulated that the increase in CD80 expression seen in active MS was causally related to disease activity and that IFN β-1b would decrease the number of

CD80-expressing cells. IFN β-1b downregulated the number of CD80$^+$ lymphocytes three- to fourfold from pretreatment values ($P < 0.01$) (Fig. 5 *A*). Patients treated with IFN β-1b comprised both active and stable forms of MS. For this reason, the mean pretreatment value falls between those for the active and stable patients in Fig. 1. The number of CD80$^+$ cells dropped in both stable and active MS after therapy (data not shown).

The number of CD86$^+$ lymphocytes was not meaningfully altered by IFN therapy (6.7±2.4% pre- vs. 4.6±1.1% posttherapy) (Fig. 5 *B*). However, the ratio of CD80$^+$/CD86$^+$ lymphocytes shifted downwards after IFN therapy. The decrease was more pronounced in active MS (5.8±2.8 pre- vs. 0.8±0.2 posttherapy, $P < 0.05$) than in stable MS (1.8±0.7 pre- vs. 1.1±0.1 posttherapy, NS). Posttreatment ratios were at or below the ratio found in controls (1.4±0.3) for both MS treatment groups.

IFN β-1b therapy increases monocyte CD86 expression. The percentage of CD80$^+$ monocytes did not change significantly after IFN β-1b therapy ($P < 0.16$), but the number of CD86$^+$ monocytes increased threefold ($P < 0.05$) (Fig. 6). The CD80/CD86 ratio on monocytes sank below control levels after IFN therapy (4.5±3.0 pre- vs. 0.6±0.2 posttherapy for all MS patients, compared with 2.0±1.3 for controls). The decrease in the CD80/CD86 ratio was more pronounced in active MS (5.2±4.4 pre- vs. 0.5±0.2 posttherapy, NS) than in stable MS (3.4±3.0 pre- vs. 0.9±0.4 posttherapy, NS). Thus, IFN β-1b therapy increases the number of CD86$^+$ monocytes and pari passu decreases disease activity.

IFN β-1b therapy reduces HLA-DR, transferrin, and IL-2 receptor expression. Since the number of CD80$^+$ lymphocytes

Table I. The Percentage of HLA-DR and Transferrin Receptor–positive MNC in MS

Subjects	Lymphocytes		Monocytes	
	HLA-DR	CD71	HLA-DR	CD71
Active MS ($n = 12$)	13.3±1.1‡	47.1±6.9‡	68.9±6.2*	58.4±6.4*
Stable MS ($n = 9$)	10.4±1.2	16.2±4.3§	61.2±7.4	23.5±11.2
Control ($n = 23$)	9.4±0.6	25.6±4.1	51.9±5.0	41.2±6.6

*vs. control, $P < 0.002$; ‡vs. control, $P < 0.05$; §vs. active MS, $P < 0.0005$.

A

% of CD80+ LYMPHOCYTES

B

% of CD86+ LYMPHOCYTES
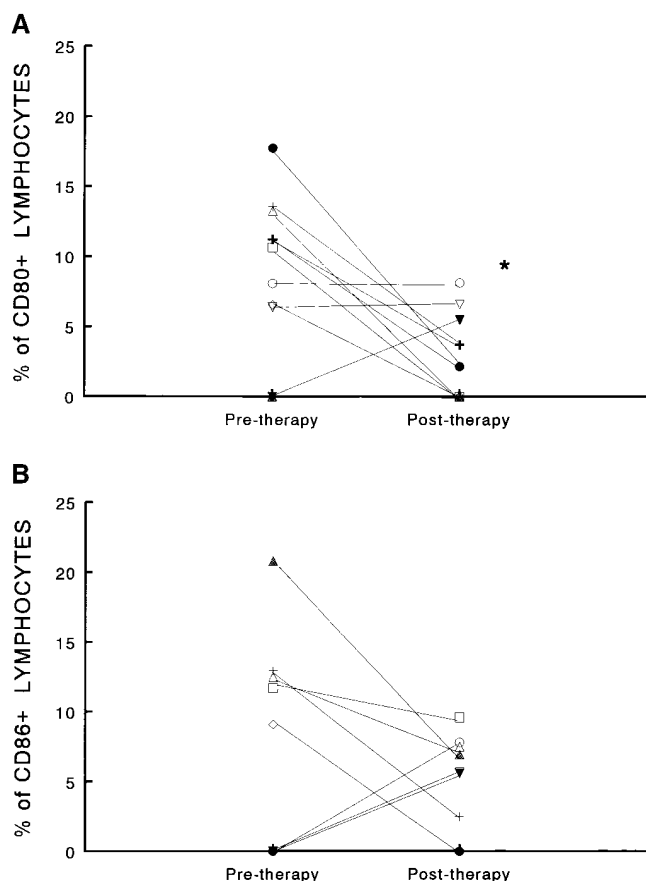
Figure 5. The percentage of CD80$^+$ and CD86$^+$ lymphocytes before and after IFN β-1b therapy. (*A*) The percentage of CD80$^+$ lymphocytes falls after IFN β-1b therapy (*$P < 0.01$; paired *t* test). (*B*) The decline in CD86$^+$ lymphocytes after IFN β-1b therapy was not significant.

A

% of CD80+ MONOCYTES

B

% of CD86+ MONOCYTES

Figure 6. (*A*) The percentage CD80$^+$ monocytes does not rise significantly after therapy. (*B*) The percentage of CD86$^+$ monocytes is increased after therapy ($P < 0.05$; paired *t* test).
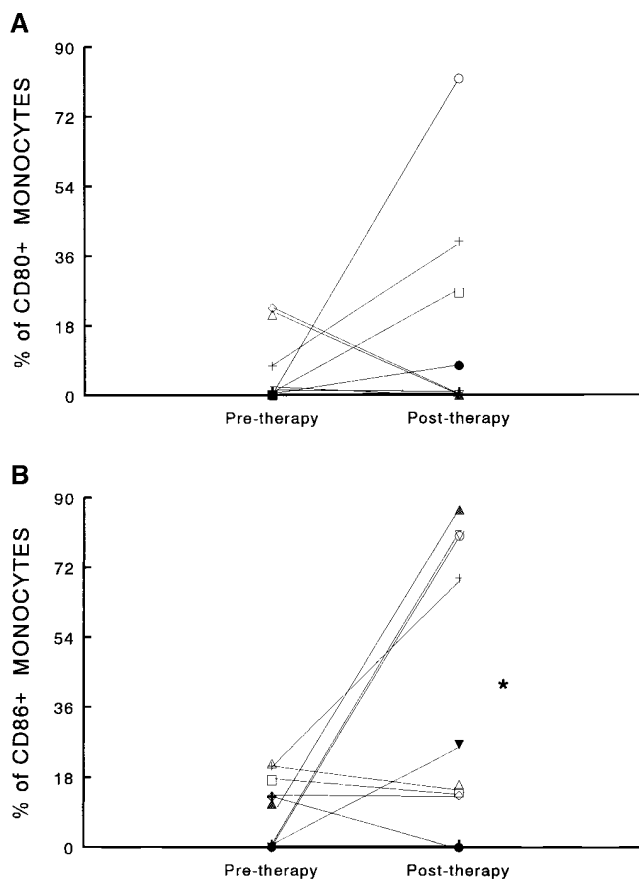
was decreased by IFN β-1b therapy, we determined whether there was a parallel decline in expression of HLA-DR, as seen for the costimulatory CD80/CD86 proteins. IFN therapy reduced the number of HLA-DR$^+$ lymphocytes by 44% (Table II), to a level below that seen in normal controls ($P < 0.005$ vs. controls in Table I). The percentage of HLA-DR$^+$ monocytes in MS patients also decreased after IFN therapy to become equivalent to control values.

After IFN β-1b therapy, the percentage of CD71$^+$ lymphocytes was reduced by 78% (Table II), to a level below that for controls ($P < 0.0008$ vs. controls in Table I). The number of CD71$^+$ lymphocytes after IFN therapy was similar to the low level observed in stable MS (Table I).

IL-2 receptor (CD25) expression increases with lymphocyte activation. There was a pronounced decrease in the number of CD25$^+$ lymphocytes after IFN β-1b therapy (17.8±7.8 pre- vs. 6.8±4.4 posttreatment, $n = 3$, $P < 0.05$). This in vivo decline in CD25 expression parallels the drop in HLA-DR$^+$ and CD80/CD86$^+$ MNC after IFN β-1b therapy (Table II and Figs. 5 and 6).

Discussion

In active MS, the increase in CD80$^+$ B cells (Fig. 1), as well as the elevated number of HLA-DR$^+$ and CD71$^+$ monocytes and

lymphocytes (Table I), results from an unknown stimulus for immune cell activation (20, 26–28). During exacerbations, IL-2, IFN-γ, TNF-α, and TNF-β appear in serum, their respective mRNAs are upregulated in MNC (29–32), and activated cells increase in the peripheral blood (20, 29, 33). CD80$^+$ and CD86$^+$ cells, as well as MHC class II$^+$ cells (34, 35), are also present in active MS plaques. CD80$^+$ (BB1$^+$) T cells are prominent among the perivenular inflammatory cells in MS lesions (19). CD80$^+$ B cells, microglia, and macrophages in plaques (17–19) could frustrate normal tolerogenic mechanisms, en-

Table II. IFN β-1b Therapy Decreases the Percentage of HLA-DR and Transferrin Receptor–positive MNC in MS

Subjects	Lymphocytes			Monocytes	
	HLA-DR	CD71	CD25	HLA-DR	CD71
	$n = 9$	$n = 7$	$n = 3$	$n = 9$	$n = 6$
Pre–IFN-β	13.1±1.5	49.5±10.5	17.8±7.8	59.7±8.4	44.2±14.0
Post–IFN-β	7.3±0.5*	10.7±1.6‡	6.8±4.4§	51.3±6.4	48.3±14.1

*vs. pre–IFN-β, $P < 0.005$; ‡vs. pre–IFN-β, $P < 0.002$; §vs. pre–IFN-β, $P < 0.05$.

hance inflammatory cell activation, and exacerbate disease. The increase of CD80$^+$ peripheral B cells and the fall in CD86$^+$ monocytes in active MS (Fig. 1), and the predominant role of CD80 over CD86 in humans (7), could well predispose to the Th1 activation (8) characteristic of exacerbations.

CD80 is a critical costimulatory molecule for development of inflammatory brain disease. It is required for induction of active EAE (8), and EAE-susceptible SJL mice express more CD80 than EAE-resistant B6 mice (16). CD80 is also needed in chronic relapsing EAE to generate the recurrent exacerbations caused by recruitment of naive T cells during epitope spreading (16). Immune cells from naive mice express more CD86 than CD80, but just before the onset of clinical EAE, spleen monocytes, T cells, and B cells all upregulate CD80. In this phase of EAE, CD80$^+$ monocytes and T cells outnumber CD86$^+$ cells (16). The CD80/CD86 ratio reverses towards baseline as EAE resolves. These findings complement our data in active MS, where the CD80/CD86 ratio is increased.

The autologous mixed lymphocyte reaction (AMLR) is increased during MS exacerbations (20, 33). The AMLR measures the proliferative response of T cells stimulated by autologous non–T cells (monocytes, dendritic, B, and NK cells) (36). CTLA4-Ig fusion protein blocks the AMLR by 30–80% (Reder, A.T., unpublished data). The increased number of CD80$^+$ B cells and monocytes in active MS may explain the enhanced costimulation and proliferation observed in this assay in MS exacerbations.

IFN β-1b therapy decreased the number of circulating CD80$^+$ lymphocytes. This could be cause or effect of disease stabilization, and several mechanisms may regulate CD80 expression in MS. IFN-β treatment induces a moderate leukopenia, possibly from reduced hematopoiesis. However, the fall in CD80$^+$ cells was selective and not from generalized lymphopenia; CD80$^+$ and CD86$^+$ peripheral lymphocytes, but not total lymphocytes, were reduced. Perhaps there is a change in cell traffic that reduces the number of circulating CD80$^+$ cells. This would require CD80$^+$ and CD86$^+$ cells to have homed from blood to some other compartment. However, the most likely target compartment in MS, the brain, has fewer inflammatory lesions during IFN-β therapy (37).

The decline in CD80$^+$ B cells could reflect a direct influence of IFN β-1b on CD80/CD86 expression, but we think this unlikely because IFN-β induces CD80/CD86 expression in vitro (Genç, K., and A.T. Reder, unpublished data). More probably, the in vivo decrease in CD80 expression is indirectly mediated, and correlates with a decline in clinical disease activity. For example, IFN-β enhances IL-10 production (38, 39), and IL-10 decreases CD80/CD86 expression (40). IFN β-1b also attenuates production of IFN-γ, which is known to induce CD80 and CD86 expression (17, 41). Although IFN-γ protein and mRNA levels are seldom elevated in MS (42), intermittent elevation of serum IFN-γ or local IFN-γ effects are possible triggers. IFN-γ administration causes exacerbations of MS (43). In addition, viral infections induce MS attacks (26, 27), superantigens released during bacterial infections reactivate EAE (28), and quinolone antibiotics (a relationship between worsening of the symptoms of MS and ciprofloxacin treatment [Reder, A.T., unpublished data]) sometimes worsen MS symptoms. All three of these activators induce Th1 cytokines, including IFN-γ, which could induce CD40, CD40L, CD80, and CD86 expression, and all three worsen inflammatory brain disease. IFN β-1b inhibits secretion of IFN-γ in vitro (44), re-duces serum IFN-γ levels in vivo (45), and tends to reduce IFN-γ mRNA (42). Thus, IFN-β could potentially attenuate production of IFN-γ or other cytokines that induce CD80 and CD86.

IFN β-1b decreases the number of CD80$^+$ B cells (Fig. 5) as well as the ratio of CD80$^+$/CD86$^+$ cells, and may thereby impede Th1 cell activation in MS, and promote anergy. Anergic T cells suppress proliferation and cytokine secretion by other T cells (46, 47). In addition, IFN β-1b therapy augments the number of CD86$^+$ monocytes (Fig. 3) which could induce counterregulatory Th2 responses (8). Finally, IFN β-1a therapy impairs ConA induction of HLA-DR and CD25 expression on lymphocytes (48); IFN β-1b may also inhibit immune reactivity by reducing the number of HLA-DR$^+$ and CD25$^+$ cells in vivo (Table II).

A decrease in CD80/CD86 expression ameliorates some inflammatory disorders. The CTLA4-Ig fusion protein blocks CD80/CD86, and in turn, causes Ag-specific T cell unresponsiveness, increased apoptosis (5), and amelioration of EAE, diabetes (49), and systemic lupus erythematosus (50). CTLA4-Ig inhibits IL-2, IFN-γ, and IL-4 production but not IL-10 gene expression (51). IL-10 inhibits CD80 expression (40) and prevents EAE (52, 53). Thus, IFN-β, IL-10, and blockade of CD80 could potentially complement each other and be used to inhibit Th1-mediated inflammation.

The increase of CD80 on small numbers of activated B cells can be central in development of autoimmune disease. B cell–deficient mice do not develop autoimmune responses to cytochrome c peptides (54). Reconstitution of these mice with as few as 300 B7$^+$ B cells elicits T cell autoimmunity. These B cells may be important APCs when low antigen concentrations prevail. Because of receptor-mediated uptake of peptides, antigen-specific B cells are up to 10,000-fold more efficient at presenting antigen compared with antigen-nonspecific APCs. Finally, B cells can process peptides differently from other APCs (54), possibly giving rise to multiple isoforms of MBP and other CNS-derived peptides in MS (55).

The number of CD80$^+$ B lymphocytes in MS shows dramatic alterations that are dependent on disease activity and on therapy with IFN β-1b. Reducing CD80 expression, or blocking its function, could potentially alter the course of MS and other chronic inflammatory diseases. CD80/CD86 expression may also be useful as a monitor of therapy and in predicting the eventual course of MS.

Note added in proof: Correale et al. (56) found that PLP-responsive T cell clones from chronic progressive MS patients were resistant to anergy induction, unlike relapsing/remitting patients and healthy controls. Although CD80/86 expression was not measured, antibodies against CD80 or CD86 restored anergy. This is further evidence of a role for nonprofessional APCs (e.g., CD80/86$^+$ T cells) in MS.

Acknowledgments

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