Expression of the Costimulatory Molecule BB-1, the Ligands CTLA-4 and CD28, and their mRNA in Inflammatory Myopathies

Ken-ya Murata and Marinos C. Dalakas

From the Neuromuscular Diseases Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

To examine if the muscle fibers in patients with inflammatory myopathies have the potential to behave as antigen presenting cells (APCs), we investigated the expression of costimulatory molecules BB-1, B7-1 (CD80), and B7-2 (CD86), and their counterreceptors, CD28 or CTLA-4 (CD152), in the muscle biopsies of patients with polymyositis (PM), PM associated with human immunodeficiency virus infection (HIV-PM), sporadic inclusion body myositis (s-IBM), dermatomyositis (DM), and normal or disease controls. The expression of the B7 family of molecules on the muscle fibers was limited to BB-1. In PM, HIV-PM, and s-IBM, but not the disease controls, the nonnecrotic, MHC-class I-expressing muscle fibers, invaded or not by CD8+ T cells, had prominent membrane expression of BB-1. Several of the BB-1-positive fibers bound strongly in a cell-to-cell contact with their CD28 or CTLA-4 ligands on the autoinvasive CD8+ T cells, as confirmed by confocal microscopy. By reverse transcription-polymerase chain reaction, the expression of CD28 and CTLA-4 was up-regulated in PM, HIV-PM, and s-IBM, but not the controls. Because the BB-1-positive fibers expressed MHC-class I antigen and bound to up-regulated counterreceptors CD28 and CTLA-4 on the autoinvasive CD8+ T cells only in PM, HIV-PM, and s-IBM, the BB-1 molecule in these diseases should have a functional role in antigen presentation and T cell differentiation. These findings complement recent studies and suggest that in PM, HIV-PM, and s-IBM the muscle fibers are not only targets of CD8+ cytotoxic T cells but may also behave as "professional" APC. (*Am J Pathol* 1999, 155:453–460)

In polymyositis (PM) and sporadic inclusion body myositis (s-IBM), CD8+ cytotoxic T cells invade muscle fibers that express MHC-class I antigen.¹ There is now strong evidence based on the T cell receptor gene rearrangement that the autoinvasive CD8+ T cells are preferentially recruited and clonally expanded *in situ* by muscle-spe-

cific autoantigens.^{2,3} This implies that the CD8+ cytotoxic T cells recognize muscle antigens presented by the MHC-class I-expressing muscle fibers. A prerequisite, however, for the muscle fibers to present an antigen and behave as antigen presenting cells (APC) is the expression of the B7 family of costimulatory molecules, BB-1, B7-1 (CD80, B7), or B7-2 (CD86, B70), on the muscle fibers and of their counterreceptors, CD28 and CTLA-4 (CD152), on the autoinvasive activated T cells. These costimulatory molecules are hallmark molecules of the APC because they are essential in maintaining self-tolerance and regulating T cell immunity.⁴

B7-1 is strongly expressed on activated microglia and infiltrating macrophages within the active lesions of multiple sclerosis.⁵ In addition, B7-1 and B7-2 are moderately to strongly expressed on the cardiac myocytes of patients with acute myocarditis,⁶ suggesting that muscle fibers have the ability to become APC. Very recently, BB-1 was found to be expressed on human myofibers and on γ -interferon-stimulated myoblasts, confirming the functional role of this molecule.⁷ To examine whether, in PM, IBM, and PM associated with human immunodeficiency virus infection (HIV-PM) the MHC-class I-expressing muscle fibers invaded by CD8+ T cells possess the functional properties of APC, we investigated the coexpression of costimulatory molecules and their ligands at the protein and mRNA levels.

Materials and Methods

Immunocytochemical Study

Fresh-frozen sections of muscle biopsy specimens from 22 patients with inflammatory myopathies (7 with PM, 5 with HIV-PM, 5 with s-IBM, and 5 with dermatomyositis (DM)) were processed for immunocytochemistry. All patients had the typical clinical, electromyographic, and histopathological features of their respective disorders.^{8,9} Control specimens were obtained from 5 patients with Duchenne muscular dystrophy (DMD) and 5

Accepted for publication April 28, 1999.

Address reprint requests to Marinos C. Dalakas, M.D., Chief, Neuromuscular Diseases Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Building 10, Rm. 4N248, 10 Center DR MSC 1382, Bethesda, MD 20892-1382. E-mail: dalakas@helix.nih.gov.

patients with morphologically normal muscle. All specimens had been stored in liquid nitrogen before the study.

Five-micron serial sections of muscle biopsies were fixed in acetone for 10 minutes at 4°C, rinsed in 0.05 mol/L Tris-buffered saline (TBS), pH 7.5, for 15 minutes, and incubated for 30 minutes with a blocking solution containing 2% bovine serum albumin (BSA) and 5% normal horse serum or 5% goat serum, as described.¹⁰ The sections were then incubated with one of the following primary mouse monoclonal antibodies: a) IgM anti-human CD80 (BB-1) (clone BB1, Pharmingen, San Diego, CA), 7.5 µg/ml in 1% BSA/TBS for 1.5 hours; b) IgG anti-human CD80 (clone L307.4, Pharmingen), 12.5 µg/ml in 1% BSA/TBS for 1.5 hours; c) IgG anti-human CD74 (clone M-B741, Pharmingen) 10.0 µg/ml in 1% BSA/TBS for 1.5 hours; d) IgG anti-human CD86 (clone BU63, Ancell, Bayport, MN), 5 µg/ml in 1% BSA/TBS for 1.5 hours; e) IgG anti-human CD28 (clone L293, Becton Dickinson, San Jose, CA), 7.5 µg/ml in 1% BSA/TBS for 1.5 hours; f) IgG anti-human CD152 (CTLA-4) (clone BNI3, Pharmingen) 5 µg/ml in 1% BSA/TBS for 1.5 hours; g) IgG anti-human HLA class I antigen (clone W6/32, Dako, Carpinteria, CA), 2 µg/ml in 1% BSA/TBS for 1 hour; h) IgG anti-N-CAM (clone MY31, Becton Dickinson), 20 µg/ml in 1% BSA/TBS for 1 hour; and i) IgG anti-human CD8 (clone SFC121Thy2D3, Coulter, Miami, FL), 5 µg/ml in 1% BSA/TBS for 1 hour. After washing for 30 minutes in TBS, the sections were incubated with biotinylated goat anti-mouse IgM (for the CD80 clone BB1) or with horse anti-mouse IgG for the others, and then with rhodamine avidin D or with fluorescein isothiocyanate (FITC) avidin D. Serial sections consecutive to those processed as described were stained with modified Gomori trichrome.

Dual and Confocal Immunofluorescence Method

To determine if the autoinvasive T cells expressed CTLA-4 and made contact with B7-expressing muscle fibers, we performed double-immunostaining using mouse monoclonal antibodies against CTLA-4 and antibodies against CD80 (BB-1) or CD8+ T cells on the same sections, as described.¹⁰ In brief, 5-µm serial sections were fixed in acetone for 10 minutes at 4°C and incubated for 30 minutes with a blocking solution containing 2% BSA and 5% normal goat serum. For double immunolocalization of BB-1 and CTLA-4, the sections were incubated with primary mouse monoclonal antibody against CD80 (BB-1), washed in TBS, and incubated with rhodamine-conjugated goat anti-mouse IgM. After rinsing in 0.05 mol/L TBS, pH 7.5, the sections were incubated with a blocking solution containing 2% BSA and 5% normal goat serum, followed by an overnight incubation with the primary mouse monoclonal antibody against CTLA-4. After washing for 30 minutes in TBS, the sections were incubated with biotinylated goat anti-mouse IgG and then with FITC avidin D. For double staining with CD8 and CTLA-4, the sections were incubated overnight with the primary mouse monoclonal antibody against CTLA-4,

followed by biotinylated goat anti-mouse IgG and FITC avidin D, as described above. After rinsing and blocking, the sections were incubated with anti-human CD8 followed by rhodamine-conjugated goat anti-mouse IgG. The slides were mounted and examined with a Zeiss photomicroscope equipped with epifluorescence optics. Because the monoclonal antibodies against CD8 and CTLA-4, both of the IgG class, and the secondary anti-IgG antibody may still cross-react with each other in spite of the blocking, we reconfirmed the results in serial sections stained separately for CD8 and CTLA-4. This was also done for the CD8 and CD28. Confocal microscopy was used to clarify the antigens expressed on the surface of the invading cells. For this purpose, the CD8+ or BB-1+ cells were visualized with rhodamine (red) fluorescence and the CTLA-4 with FITC (green) fluorescence on the same sections, using a Zeiss laser confocal system (Zeiss LSM 410 laser scanning microscope).

Preparation of RNA

Total RNA was extracted using TRIZOL (Gibco BRL, Grand Island, NY). Frozen muscle biopsy sections (10 sections, 20 µm thick) were homogenized in a total volume of 0.2 ml of TRIZOL and the extracted RNA was precipitated per manufacturer's instructions. RNA was suspended in 12 µl of RNase-free water, heated for 10 minutes at 56°C, and stored at -80°C until use.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

The cDNA was prepared using a GeneAmp RNA PCR kit (Perkin-Elmer, Roche Molecular Systems, Branchburg, NJ) with 1.0 µg of total RNA in a final volume of 20 µl per the manufacturer's instructions. Before synthesis of the cDNA with random primers, 10 units of RNase-free DNase I (Boehringer Mannheim, Indianapolis, IN) was added per microgram of RNA, and the reaction mixture was incubated for 30 minutes at 37°C and for 5 minutes at 95°C. For amplification of the cDNA, 2.5 µl of the RT reaction mixture was used for each 50 µl of the PCR reaction. Each PCR mixture contained the standard components of 1.5 mmol/L MgCl₂ and 50 pmol of CTLA-4 or CD28 primers. As an internal control for RNA quantification and standardization of the amplified RNA products, we used the primer for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a constitutive and moderately expressed enzyme.

The CTLA-4, CD28, and GAPDH primers were designed using published cDNA sequences¹¹⁻¹³ (Genosys Biotechnologies, The Woodlands, TX) as follows: CTLA-4 forward, 5'-AGTATGCATCTCCAGGCAAAGC-3' (exon 1); CTLA-4 reverse, 5'-CCAGAGGAGGAAGTCAGAATCTG-3' (exon 2); CD28 forward, 5'-GTTTGAGTGCCTTGATCATGTGC-3' (exon 1); CD28 reverse, 5'-GGCGATCTGCTTCACCAAATC-3' (exon 2); GAPDH forward, 5'-TGAAGGTCTGGAGTCAACGGATTTGG-3' (exons 2-3); and GAPDH reverse, 5'-GTTTACACCCATGACGAACATGG-3' (exon 6). Both sets of primers spanned at least one intron

in their respective genomic sequence, resulting in amplified products of 316 bp for CTLA-4, 238 bp for CD28, and 398 bp for GAPDH. After an initial 3-minute period at 95°C, the PCR reaction mixtures were incubated for 40 cycles, unless otherwise stated, using a Perkin Elmer Model 480 thermocycler at 95°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute, and a final extension of 72°C for 7 minutes. The amplified products (12.5 μ l) were analyzed with a 3% gel electrophoresis and the bands were visualized with ethidium bromide (0.5 μ g/ml). Fluorescent bands were recorded on Polaroid Type 57 film (Polaroid, Cambridge, MA). RT-PCR analysis was done at least twice on every patient.

Polaroid photographs of ethidium bromide-stained gels were digitized into 512 $\times$ 512-pixel gray-scale images. The amount of nucleic acid, determined by densitometric analysis of the dots, was proportional to the logarithm of the optic density. Analysis was performed using NIH Image 1.6 program provided by the Scientific Computing Resource Center at the National Institutes of Health (Bethesda, MD).

Results

Immunohistological Study

B7-family

Among the B7 family of molecules, only the BB-1 was strongly and specifically expressed on the muscle fibers, but not on the endomysial inflammatory cells (Figure 1). In contrast, B7-1 and CD74 were undetectable on the muscle fibers, but they were present on the surface of the inflammatory mononuclear cells (Figure 1).

There were two patterns of BB-1 expression. One involved both the membrane and the cytoplasm (Figure 1) of the regenerating, basophilic, and N-CAM-positive fibers in PM, HIV-PM, s-IBM, and DM and some of the small fibers in DMD, but not the normal controls. Up to 90% of the N-CAM-positive regenerating fibers in PM, HIV-PM, s-IBM, and DM were BB-1-positive, compared to only 30% of the same fibers in DMD, where the immunostaining was also weaker. Some of the muscle fibers with rimmed vacuoles in s-IBM and the N-CAM-positive regenerating fibers within the perifascicular regions in DM were also BB-1-positive (not shown).

The second and more important pattern of BB-1 expression was in the membrane of the nonnecrotic but MHC-class I-expressing muscle fibers in PM, HIV-PM, and s-IBM (Figures 2 and 3), but not in DMD, DM, or the normal controls. The BB-1 expression was not limited to the fibers invaded by the CD8⁺ T cells, but was also present on several healthy, MHC-class I-expressing muscle fibers remote from the areas of inflammation, in a pattern identical to MHC-class I (Figure 3). Such a BB-1-positive pattern on the healthy, MHC-class I-positive fibers was found only in PM, HIV-PM, and s-IBM but not in DM, DMD, or the normal controls.

Figure 1. Transverse serial frozen sections of a muscle biopsy specimen from a patient with HIV-PM, stained with monoclonal antibodies against BB-1 (A), CD74 (B), and B7-1 (C). Note that BB-1 is expressed only on the muscle fibers but not on the surrounding lymphoid cells. Strong CD74 (B) and very weak B7-1 (C) expression is detected on the surface of some autoinvasive lymphocytes. Original magnification, $\times 400$; scale bar: 50 μ m.

Counterreceptors

The T cells invading the BB-1-positive muscle fibers in PM, HIV-PM, and s-IBM, but not in DMD, expressed CD28 (Figure 2) and, more consistently, CTLA-4 (Figure 3). Most of the autoinvasive CD28- or CTLA-4-positive cells were CD8⁺ cytotoxic T cells, as confirmed with double confocal microscopy and with serial sections (Figures 2, 3, and 4). The CD8⁺ T cells in DMD were negative for CD28 or CTLA-4, even when found around the BB-1-positive regenerating muscle fibers (Figure 5).

Figure 2. Transverse serial frozen sections of a muscle biopsy specimen from a patient with s-IBM, stained with Gomori trichrome (A) and with monoclonal antibodies against BB-1 (B), MHC-class I antigen (C), CD8 (D), and CD28 (E). The nonnecrotic (*) fibers (A) invaded by CD8⁺ T cells (D) express MHC-class I antigen (C) and BB-1 (B). Non-necrotic fibers, even if not in contact with the T cells (**), express BB-1 only on the cell membrane. The necrotic, degenerating, and small fibers express BB-1 throughout the cell. Many of the autoinvasive cells express the CD28 antigen (E). Original magnification, $\times 400$; scale bar, 25 μm .

Figure 3. Transverse serial frozen sections of a muscle biopsy specimen from a patient with HIV-PM, stained with Gomori trichrome (A) and with monoclonal antibody against BB-1 (B), MHC-class I antigen (C), CD8 (D), and CTLA-4 (E). The nonnecrotic fiber (*) invaded by CD8+ T cells (A and D), as well as healthy fibers remote from the inflammatory reaction express both MHC-class I antigen (C) and BB-1 (B). The pattern of BB-1 expression is similar to MHC-class I. Several of the autoinvasive T cells also express CTLA-4 (E). Original magnification, $\times 400$; scale bar: 50 μm .

Figure 4. Confocal microscopic images of a muscle biopsy from a patient with HIV-PM, dually immunostained for BB-1 (red) and CTLA-4 (green) (A), or for CD8 (red) and CTLA-4 (green) (B). A and B are serial sections. Areas with double staining are shown in yellow-orange due to the superimposition of green-to-red fluorescence. A: The CTLA-4 expression is restricted to the surface of the lymphocytes (green) invading or surrounding the BB-1-positive muscle fibers (red). A cell-to-cell contact between BB-1 and CTLA-4 is noted. No BB-1 staining is noted on the lymphocytes or CTLA-4 staining on the muscle fibers. B: Most of the CD8-positive autoinvasive cells express the CTLA-4 antigen, shown in yellow. Original magnification, $\times 800$; scale bar, 10 μm .

RT-PCR

The amounts of extracted CTLA-4, CD28, and GAPDH mRNA were studied with increasing numbers of PCR cycles. All the amplified products were compared after 40 cycles, which fell in the linear portions of the curve. As shown in Figure 6A, strong CTLA-4 mRNA and very faint CD28 mRNA expression was amplified only in the muscles of patients with PM, HIV-PM, and s-IBM, but not in

Figure 5. Transverse serial frozen sections of a muscle biopsy specimen from a patient with DMD immunostained with monoclonal antibodies against CD8 (A), CTLA-4 (B), and CD28 (C). Note that the autoinvasive CD8+ T cells are negative for CTLA-4 (B) and CD28 (C). Original magnification, $\times 400$; scale bar, 50 μm .

those of the controls. The amount of GAPDH was consistent in all.

An attempt was made to semiquantify the CTLA-4 or CD28 in reference to the GAPDH amplified in the same samples by scanning each band. Although the standard deviation differed among patients, there was no apparent difference in the mean amount of CTLA-4 mRNA expression between PM, HIV-PM, and s-IBM (Figure 6B). The

Figure 6. A: RT-PCR analysis of mRNA for CTLA-4 (316 bp), CD28 (238 bp), and control enzyme GAPDH (398 bp) in the muscle biopsies of patients with PM (lanes 1–3), HIV-PM (lanes 4–6), and s-IBM (lanes 7–9), and controls (lanes 10–12). M indicates the size marker. Strong GAPDH and CTLA-4 mRNA expression and weak CD28 mRNA expression is seen in PM, HIV-PM, and s-IBM. Neither CTLA-4 nor CD28 mRNA expression was amplified in the normal control specimens. In s-IBM, only a very faintly amplified amount of the CD28 was observed. B: Semiquantitative expression of mRNA for CTLA-4 and CD28 in the studied muscle biopsy specimens. No apparent difference in the mean amount of CTLA-4 mRNA is noted in PM, HIV-PM, and s-IBM. The amount of amplified mRNA for CD28 in the muscles of s-IBM patients was very small.

expression of CTLA-4 mRNA was higher than the CD28 in all the patients, confirming the immunohistochemical findings.

Discussion

The study showed that in patients with PM, HIV-PM, and s-IBM, but not the disease controls, the main costimulatory molecule BB-1, but not B7-1 or B7-2, is specifically expressed on the healthy, MHC-class I-expressing muscle fibers. Further, the BB-1-positive muscle fibers in these diseases made cell-to-cell contact with their counterreceptors CD28 and CTLA-4 on their autoimmune CD8+ T cells. Because these costimulatory molecules and their ligands are the prerequisites for antigen presentation, the findings suggest that in PM, HIV-PM, and s-IBM, the muscle fibers have the functional properties of APC. The observation supports recent *in vivo* and *in vitro* data showing that BB-1 is expressed on the muscle fibers of patients with myositis, is up-regulated in human myoblasts by cytokines, and plays an important role in the immunobiology of the muscle.⁷

The expression of BB-1 in the muscle was also observed in the cytoplasm of N-CAM-positive regenerating fibers in all the diseases studied, including DM and DMD. However, the CD28 and CTLA-4 ligands were present on the autoimmune CD8+ T cells only in PM, HIV-PM, and s-IBM. The findings collectively indicate that the cytoplasmic expression of BB-1 in regenerating muscle fibers is not related to an APC function, but rather represents a developmental molecule, similar to N-CAM, that is further up-regulated by cytokines, as has been shown for the myoblasts *in vitro*.^{7, 14}

Based on recent data, the B7 family of costimulatory molecules on the APC, includes three distinct forms, the

B7-1 (CD80), B7-2 (CD86 or B70), and BB-1.^{7, 15, 16} Although cDNA for the BB-1 antigen has not been isolated, BB-1 is distinct from B7-1 because it is expressed on B7-1-negative cells.¹⁷ The report that BB-1 also reacts with the CD74, an MHC-class II-associated invariant chain, and represents an epitope of B7-1¹⁶ does not appear to be relevant to the muscle because the antibodies against CD74 did not stain the muscle fibers. Only the T cells were CD74-positive (Figure 1).

In human myoblasts, blockage of the BB-1 molecule inhibits the myoblasts' capacity for antigen presentation in the presence of CTLA-4, suggesting that BB-1 is a functional CTLA-4-binding costimulatory molecule.⁷ These functional studies are supported by the molecular interaction we noted between BB-1 and CTLA-4 and they are consistent with the observation that an APC must express B7 molecules, including BB-1, and bind to counterreceptors on T cells for T cell activation, differentiation, and termination.^{15, 17, 18} On this basis, the present findings support the view that in PM, HIV-PM, and s-IBM, even if the antigen is unknown, the muscle fibers appear to possess the necessary requirements for antigen presentation, because collectively a) they express costimulatory molecules and make close cell-to-cell contact with their counterreceptors on the autoimmune CD8+ T cells (Figures 2, 3, and 4); b) the T cell receptor genes of their autoimmune T cells are rearranged and the CD3 region has a unique sequence²⁻³; c) the MHC-class I antigen is up-regulated on the BB-1-expressing muscle fibers (Figures 2 and 3); d) there is up-regulation of adhesion molecules LFA-1 on T cells and ICAM-1 on the muscle fibers^{19, 20}; and e) there is increased expression of various cytokines, which play a pivotal role in inducing BB-1 and MHC-class I.⁷

Although the costimulatory molecules of the B7 family are associated mostly with CD4+ T cells in response to antigens presented by MHC-class II, the cytotoxic CD8+ T cells can be also stimulated by peptide antigens presented by "professional" APCs in association with MHC-class I molecules. Such CD8+ cytotoxic T cells do not require exogenous help from the CD4+ T cells and are referred to as helper-independent cytotoxic T cells.^{21, 22} These cells encounter a peptide antigen complexed with MHC-class I molecule on the surface of APC when they receive signals for differentiation either from the costimulatory molecules expressed by the APC or in response to cytokines up-regulated by the costimulators.²² In PM, HIV-PM, and s-IBM, not only the costimulatory molecule BB-1 and its counterreceptors, but also proinflammatory cytokines such as interleukin-6, interferon γ (IFN- γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor- α , which are up-regulated within the muscle microenvironment,^{3, 23} can provide the necessary signals on the muscle fibers for antigen presentation to T cells.

The protein and mRNA expression of CD28 on the CD8+ T cells was much weaker than the CTLA-4 expression, probably because, *in vitro*, the CTLA-4 is highly expressed during the activation of T cells and binds to the B7 family with a 10- to 100-fold higher affinity than the CD28.²⁴ Because CTLA-4-positive cells are functionally active when the function of CD28-positive cells is low, these two T cell subpopulations may provide complementary signals to each other.²⁵ Even though the exact costimulatory role of these two ligands in tissues is not yet clear, their degree of expression on the autoinvasive T cells in PM, HIV-PM, and s-IBM muscles may vary according to the stage and activity of the disease.

Our findings provide evidence that in PM, HIV-PM, and s-IBM, the muscle fibers express the costimulatory molecules seen in the cells with the APC phenotype. Given the *in vitro* data on the myoblasts,⁷ it appears that in these diseases the myofibers are not only targets of the CD8+ cytotoxic T cells, but also possess the properties necessary to function as "professional" APC.

References

- Emslie-Smith AM, Arahata K, Engel AG: Major histocompatibility complex class I antigen expression, immunolocalization of interferon subtypes, and T cell-mediated cytotoxicity in myopathies. *Hum Pathol* 1989, 20:224–231
- Bender A, Ernst N, Iglesias A, Dornmair K, Wekerle H, Hohlfield R: T cell receptor repertoire in polymyositis: clonal expansion of autoregressive CD8+ T cells. *J Exp Med* 1995, 181:1863–1868
- Dalakas MC: Molecular immunology and genetics of inflammatory muscle diseases. *Arch Neurol* 1998, 55:1509–1512
- Reiser H, Stadelcker MJ: Costimulatory B7 molecules in the pathogenesis of infectious and autoimmune diseases. *N Engl J Med* 1996, 335:1369–1377
- De Simone R, Giampaolo A, Giometto B, Gallo P, Levi G, Peschle C, Aloisi F: The costimulatory molecule B7 is expressed on human microglia in culture and in multiple sclerosis acute lesions. *J Neuropathol Exp Neurol* 1995, 54:175–187
- Seko Y, Takahashi N, Ishiyama S, Nishikawa T, Kasajima T, Hiroe M, Suzuki S, Ishiwata S, Kawai S, Azuma M, Yagita H, Okumura K, Yazaki Y: Expression of costimulatory molecules B7-1, B7-2, and CD40 in the heart of patients with acute myocarditis and dilated cardiomyopathy. *Circulation* 1998, 97:637–639
- Behrens L, Kerschensteiner M, Misgeld T, Goebels N, Wekerle H, Hohlfield R: Human muscle cells express a functional costimulatory molecule distinct from B7.1 (CD80) and B7.2 (CD86) *in vitro* and in inflammatory lesions. *J Immunol* 1998, 161:5943–5951
- Dalakas MC: Polymyositis, dermatomyositis, and inclusion-body myositis. *N Engl J Med* 1991, 325:1487–1498
- Dalakas MC: Immunopathogenesis of inflammatory myopathies. *Ann Neurol* 1995, 37(S1):S74–S86
- Illa I, Dinsmore S, Dalakas MC: Immune-mediated mechanisms and immune activation of fibroblasts in the pathogenesis of eosinophilia-myalgia syndrome induced by L-tryptophan. *Hum Pathol* 1993, 24:702–709
- Dariavach P, Mattéi MG, Golstein P, Lefranc MP: Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. *Eur J Immunol* 1988, 18:1901–1905
- Lee KP, Taylor C, Petryniak B, Turka LA, June CH, Thompson CB: The genomic organization of the CD28 gene implications for the regulation of CD28 mRNA expression and heterogeneity. *J Immunol* 1990, 145:344–352
- Ercolani L, Florence B, Denaro M, Alexander M: Isolation and complete sequence of a functional human glyceraldehyde-3-phosphate dehydrogenase gene. *J Biol Chem* 1988, 263:15335–15341
- Schubert W, Zimmermann K, Cramer M, Starzinski-Powitz A: Lymphocyte antigen Leu-19 as a molecular marker of regeneration in human skeletal muscle. *Proc Natl Acad Sci USA* 1989, 86:307–311
- Boussiotis VA, Freeman GJ, Gribben JG, Daley J, Gray G, Nadler LM: Activated human B lymphocytes express three CTLA-4 counterreceptors that costimulate T-cell activation. *Proc Natl Acad Sci USA* 1993, 90:11059–11063
- Freeman GJ, Cardoso AA, Boussiotis VA, Anumanthan A, Groves RW, Kupper TS, Clark EA, Nadler LM: The BB1 monoclonal antibody recognizes both cell surface CD74 (MHC class II-associated invariant chain) as well as B7-1 (CD80), resolving the question regarding a third CD28/CTLA-4 counterreceptor. *J Immunol* 1998, 161:2708–2715
- Nickoloff BJ, Mitra RS, Lee K, Turka LA, Green J, Thompson C, Shimizu Y: Discordant expression of CD28 ligands, BB-1, and B7 on keratinocytes *in vitro* and psoriatic cells *in vivo*. *Am J Pathol* 1993, 142:1029–1040
- Simon JC, Dietrich A, Mielke V, Wuttig C, Vanscheidt W, Linsley PS, Schöpf E, Sterry W: Expression of the B7/BB1 activation antigen and its ligand CD28 in T-cell-mediated skin diseases. *J Invest Dermatol* 1994, 103:539–543
- De Bleecker JL, Engel AG: Expression of cell adhesion molecules in inflammatory myopathies and Duchenne dystrophy. *J Neuropathol Exp Neurol* 1994, 53:369–376
- Bartocioni E, Gallucci S, Scuderi F, Ricci E, Servidei S, Broccolini A, Tonali P: MHC class I, MHC class II and intercellular adhesion molecule-1 (ICAM-1) expression in inflammatory myopathies. *Clin Exp Immunol* 1994, 95:166–172
- Harding FA, Allison JP: CD28–B7 interactions allow the induction of CD8+ cytotoxic T lymphocytes in the absence of exogenous help. *J Exp Med* 1993, 177:1791–1796
- Abbas AK, Lichtman AH, Pober JS: Cellular and Molecular Immunology. 3rd Ed. Philadelphia, WB Saunders, 1997, pp 278–296
- Lundberg I, Brengman JM, Engel AG: Analysis of cytokine expression in muscle in inflammatory myopathies, Duchenne dystrophy, and non-weak controls. *J Neuroimmunol* 1995, 63:9–16
- Lane P: Regulation of T and B cell responses by modulating interactions between CD28/CTLA4 and their ligands, CD80 and CD86. *Ann NY Acad Sci* 1997, 815:392–400
- Lenschow DJ, Walunas TL, Bluestone JA: CD28/B7 system of T cell costimulation. *Annu Rev Immunol* 1996, 14:233–258