Histol Histopathol (1996) 11: 97-102

Histology and Histopathology

Major histocompatibility complex expression in muscle of rats with graft-versus-host disease

Y. Ohba[1], Y. Fujikura[2], T. Sawada[2], N. Tokuda[2], M. Morimatsu[1] and T. Fukumoto[2]
Departments of [1]Neurology and [2]Anatomy, Yamaguchi University School of Medicine, Ube, Japan

Summary. Immunohistochemical examination of rat skeletal muscle during graft-versus-host disease (GVHD), a systemic immune reaction, was performed to investigate specific immune reactivities focusing on major histocompatibility complex (MHC) expression and inflammatory cell infiltration of skeletal muscle during a systemic immune reaction. MHC class II expression and inflammatory cell infiltration did not increase. MHC class I was expressed along the contour of muscle fibres, and most strongly expressed by the cells which were distributed throughout the endomysium and perimysium. Seventy-six percent of these MHC class I^+ cells carried endothelial cell-markers, while 24% of them did not. The latter cells were revealed not to be inflammatory cells such as lymphocytes, granulocytes or macrophages when examined by immunostaining using several exudate-cell markers. Neither were they myosatellite cells because they were located outside the basement membrane. These results may be useful for considering animal models of inflammatory myopathies such as polymyositis and dermatomyositis.

Key words: Rat, Skeletal muscle, Graft-versus-host disease, Major histocompatibility complex, Immunohistochemistry

Introduction

Recently, the importance of immunological investigation of muscle has been noticed in some clinical cases (Arahata and Engel, 1984; Karpati et al., 1988; Emslie-Smith et al., 1989; Bartoccioni et al., 1994). As far as the whole immune system is regarded, muscle tissues have been almost ignored in the field of immune response. In health, MHC class I antigen is not detected in human skeletal muscle, although its expression and lymphocyte infiltration become evident during polymyositis and dermatomyositis which are considered to be autoimmune diseases (Arahata and Engel, 1984; Karpati et al., 1988; Emslie-Smith et al., 1989; Bartoccioni et al., 1994). Thus, some descriptions about inflammatory or autoimmune disease in muscle raises the necessity of more detailed study on immunological phenomena occurring in muscle tissues (Hohlfeld and Engel, 1994). Immunological aspects of muscle tissues, such as behaviour of immunopotent cells, expression of major histocompatibility complex (MHC) products in muscle tissue, and microenvironment as a place of immune response, are necessary to understand the pathogenetic mechanism of various muscle diseases. Expression of MHC products and changes in the distribution of immunopotent cells occur in representative immune tissues, such as epidermal cells and gut epithelium (Barclay and Mason, 1982; Lampert et al., 1982; Mason et al., 1982; Bland and Whiting, 1992), even in the central nervous system (Hickey and Kimura, 1987; Kajiwara et al., 1991), and such a phenomenon has been considered to induce immune response and inflammation within these tissues. However, there exist few reports about experimental study, especially *in vivo*. Here we examined the expression of MHC class I and immunopotent cells in muscle tissue under graft-versus-host disease (GVHD), a strong systemic immune reaction associated with allogeneic recognition which affects the expression of MHC products in various organs.

Offprint requests to: Dr. Yukio Ohba, Department of Neurology, Yamaguchi University School of Medicine, Ube 755, Japan

Materials and methods

Animals

Inbred Lou/M ($RT1^u$), Lewis ($RT1^l$) and DA ($RT1^a$) strain female rats, aged 8 weeks were housed 5 to a plastic cage and maintained in a conventional fashion with food and water *ad libitum*. Lewis rats were used only to investigate the origin of MHC class I antigens induced during GVHD.

Antibodies in direct immunostaining

We labelled Cy3 (Southwick et al., 1990) to HAM2 (Fukumoto et al., 1984), and used it in double immunofluorescence staining.

Antibodies in indirect immunostaining

We used murine monoclonal antibodies directed against rat MHC-encoded antigens and leukocyte surface markers as shown in Table 1. HAM2 (Fukumoto et al., 1984) and OX18 (Fukumoto et al., 1982) are against MHC class I antigen. I1.69 cross-reacts with Lewis specific MHC class I, and not with that of DA and Lou/M strain rats (Kimura and Willson, 1984). OX6 is against MHC class II antigen (McMaster and Williams, 1979). OX3 reacts to MHC class II antigen of Lou/M, Lewis and AO, but not of DA, BN and PVG/c rats (McMaster and Williams, 1979). OX19 is against CD5 (Dallman et al., 1984), OX8 is against CD8 (Brideau et al., 1980), OX35 is against CD4 (Jefferies et al., 1985) and W3/13 against leukosialoglycoprotein, which has recently been named CD43 (Williams et al., 1977). ED1, ED2 and ED3 recognize certain subsets of macrophages, although those subsets have not been well characterized (Dijkstra et al., 1985). HIS52 reacts to pan-endothelial cells (RECA-1 antigen) (Duijvestijn et al., 1992), and was kindly donated by Prof. P. Nieuwenhuis (Department of Histology and Cell Biology, University of Groningen, Netherlands). Monoclonal anti-rat ICAM-1 is against CD54 (Tamatani and Miyasaka, 1990). In addition to these monoclonal antibodies, anti-laminin rabbit polyclonal antibody (Medac, Gessellschaft für klinische Spezialpräparate mbH, Hamburg) was used.

As secondary antibodies, horseradish peroxidase (HRP)- or fluorescein isothiocyanate (FITC)-conjugated goat $F(ab')_2$ anti-mouse IgG (Cappel Laboratories Inc., West Chester, PA, USA) and rhodamine isothiocyanate (RITC)-conjugated goat anti-rabbit IgG (Amersham, UK) were used at the concentration of 10-20 µg/ml.

Table 1. Mono- and polyclonal antibodies used in this experiment.

ANTIBODY	CLASS AND SUBCLASS	RECOGNITION
HAM 2	TgG2a	MHC clas I (Fukumoto et al., 1984)
OX18	IgG1	MHC class I (Fukumoto et al., 1982)
I1.69	IgG2c	MHCclass I (positive for Lewis, negative for DA and Lou/M) (Kimura and Willson, 1984)
OX6	IgG1	MHC class II, Ia-A
OX3	IgG1	MHC Class II (positive for Lou/M, Lewis and AO, negative for DA) (McMaster and Williams, 1979)
OX19	IgG1	CD5, peripheral T, thymocyte (Dallman et al., 1984)
OX8	IgG1	CD8 (Brideau et al., 1984)
OX35	IgG2a	CD4 (Jefferies et al., 1985)
W3/13	IgG1	Leukosialoglycoprotein (CD43), peripheral T, thymocytes (Williams et al., 1977)
ED1	IgG1	Macrophages (Dijkstra et al., 1985)
ED2	IgG2a	Macrophages (Dijkstra et al., 1985)
ED3	IgG2a	Macrophages (Dijkstra et al., 1985)
HIS52	IgG1	Pan-endothelial cells (Duijvestijn et al., 1992)
Anti-ICAM-1	IgG1	CD54 (Tamatani et al, 1990)
Anti-laminin		Glycoprotein in basement membrane

Preparation of lymphocytes

Cervical and mesenteric lymph nodes of Lou/M (or Lewis) rats were teased gently on a stainless steel mesh, and cells were suspended in saline after washing 3 times. Viability of cells was checked by trypan blue exclusion, showing that more than 90% of cells were viable.

Induction of graft-versus-host disease (GVHD)

Lymphocytes ($5x10^7$) from Lou/M (or Lewis) rats were injected intravenously into DA rats after sublethal irradiation (6Gy, MBR-1520R, Hitachi Medical. Co., Ltd. Tokyo, Japan). The development of GVHD was confirmed by a combination of several clinical signs; weight loss, decreased activity, diarrhoea, alopecia, irritability, and erythema in the ears and paws. The rats were sacrificed and autopsies were performed on days 5 (n=5) or 10 (n=22) after lymphocyte injection. Normal DA rats of the same age were examined as controls.

Paraffin sections

Muscle tissues for specimens were harvested from triceps muscle of arm. Skin from ears and spleen were also harvested to examine histological changes, such as infiltration of inflammatory cells and changes of connective tissue components following GVHD. Those specimens were fixed in Carnoy's fixative, paraffin embedded, and sections were stained with haematoxylin and eosin.

Immunoperoxidase staining procedure

Frozen sections were air-dried for 30 min and fixed in methanol at 4 °C for 10 min. After rinsing in PBS, the specimens were exposed to 100 µl of primary antibody for 60 min at room temperature. After further rinsing in PBS, the sections were then incubated with HRP-conjugated goat $F(ab')_2$ anti-mouse IgG for 60 min at 4 °C. After another rinse in PBS, HRP-bound sites were developed with 0.05% diaminobenzidine tetrahydrochloride in 0.025M Tris buffer (pH 7.6) containing 0.01% H_2O_2.

Immunofluorescence staining procedure

Methanol-fixed sections were reacted with primary antibody for 60 min at room temperature. After rinsing in phosphate-buffered saline (PBS), the sections were incubated with FITC-conjugated goat $F(ab')_2$ anti-mouse IgG for 60 min at 4 °C. Double-immunofluorescence staining was performed using 1) OX18 and

anti-laminin rabbit antibody followed by FITC-conjugated goat $F(ab')_2$ anti-mouse IgG and RITC-conjugated goat anti-rabbit IgG, 2) HIS52 followed by FITC-conjugated $F(ab')_2$ anti-mouse IgG and Cy3-labelled HAM2. FITC, RITC and Cy3 were detected using a fluorescence microscope (Nikon XF-EF, Tokyo, Japan). As controls of first antibodies, normal rabbit serum was used.

Results

Body weight changes and clinical signs to determine GVHD

The recipients gradually lost weight (day 0; 125.8±3.4, day 2; 116.1±3.5, day 4; 118.1±2.9, day 6; 111.4±3.4, day 8; 109.1±2.7; day 10; 105±2.5 g: mean±SE) and their skin developed erythema seen best in the ears and paws on days 7-9 (data not shown).

Microscopical findings

In both skin and muscle, no remarkable infiltration of inflammatory cells, tissue necrosis or changes of connective tissue components were detected on days 5 and 10. In the spleen, nucleated cells had increased diffusely on day 5, particularly in the red pulp, so that the typical structure of white pulp, red pulp and marginal zones became less distinct. This change in the spleen was most pronounced on day 10 (data not shown).

Expression of MHC antigens in the skin

In normal rat skin, MHC class II antigen was expressed mainly at the germinative layer of the epidermis and hair follicle. In GVHD rat skin, MHC class II antigen expression became strong on day 5 and much stronger on day 10. OX3 (MHC class II of Lou/M and Lewis but not DA) antigen was detected in neither normal nor GVHD rat skin. MHC class I antigen was not clearly detected in normal rat skin whereas it was present at low levels in the germinative layer of GVHD rat skin on day 5. In GVHD rat skin, this antigen was detected at high levels in the germinative layer and hair follicles on day 10 (data not shown).

Expression of MHC antigens in GVHD rat muscle

MHC class I antigen was strongly detected on cells distributed in the endomysium and perimysium in GVHD rat muscle on day 5 and more strongly on day 10 (Figs. 1B,C), whereas it was rarely detected in normal rat muscle (Fig. 1A). These MHC class I-positive (MHC class I^+) cells were small cells which appeared to be either fusiform, round or carrying dendrites. Similar results were obtained by immunofluorescence (Fig. 2A). MHC class II antigen was demonstrated on a few cells like macrophages existing in the endomysium and perimysium in both normal and GVHD rat muscle. The frequency of MHC class II^+ cells in GVHD rat muscle was similar to that in normal rat muscle. MHC class II antigen was not detected on MHC class I^+ cells nor along the contour of muscle fibres in normal or GVHD rat muscle.

We used Lewis rats as donors to investigate the origin of MHC class I antigens induced during GVHD. I1.69-positive ($I1.69^+$) cells (Lewis origin cells) were not detected in the muscle of rats suffering from GVHD induced by injecting Lewis lymphocytes into DA rats, although these cells were found to be distributed

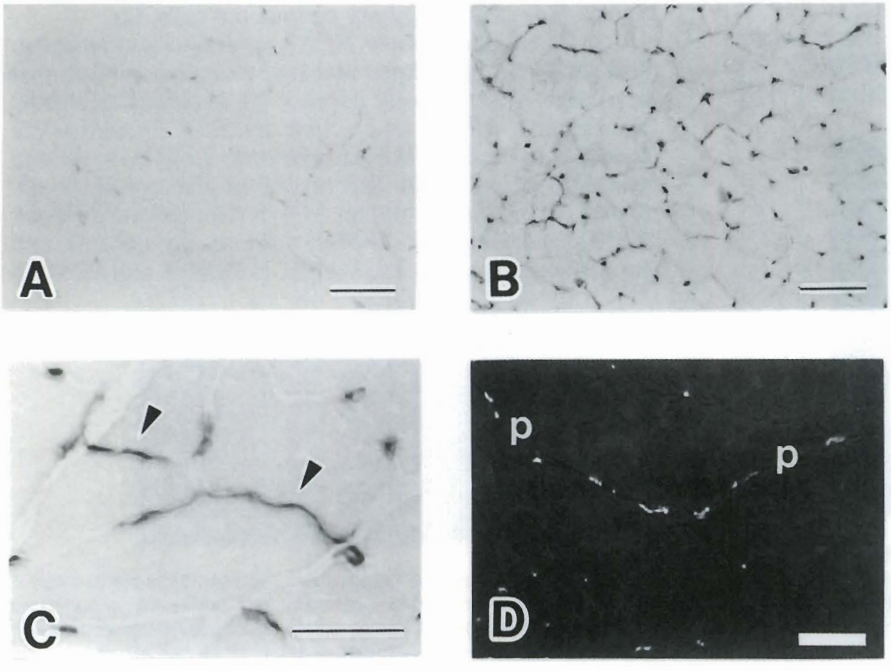

Fig. 1. Normal and GVHD rat muscles are stained by the immunoperoxidase technique with OX18 antibody **(A-C)**. MHC class I (OX18) antigen is detected along the contour of muscle fibres and on many small cells distributed in both the endomysium and perimysium in GVHD rat muscle on day 10 (B, C), while this antigen is rarely detected in normal rat muscle (A). Arrowheads show some MHC class I-positive cells (C). Immunofluorescence staining with ED2 in GVHD rat muscle **(D)**. $ED2^+$ cells are obvious along the perimysial sites. p: perimysium. Scale bar: 50μm (A, B), 25 μm (C) and 100 μm (D).

diffusely in the spleen (data not shown). There were no $OX3^+$ cells (Lewis origin cells) in normal or GVHD muscle (data not shown).

Cell infiltration in GVHD rat muscle

We attempted to detect inflammatory cells exudated in GVHD rat muscle. Inflammatory-cell markers (CD4, CD5, CD8 and leukosialoglycoprotein) were rarely detected and only in perivascular sites in GVHD rat muscle, and there were some dendritic $OX35^+$ cells in endomysium and perimysium. Infiltration of macrophages was examined by the markers (ED1, ED2 and ED3). In normal muscle, $ED2^+$ cells occurred frequently, while $ED1^+$ and $ED3^+$ cells appeared less frequently (data not shown). In GVHD rat muscle, $ED2^+$ and $ED3^+$ cells were increased in number compared to normal muscle, while $ED1^+$ cells were detected less frequently. There was an increased number of $ED2^+$ in GVHD muscle, predominantly localized along the perimysial sites (Fig. 1D). Similar results were also obtained by immuno-peroxidase staining.

ICAM-1 expression in rat muscle

Expression of ICAM-1, which was rarely seen in normal muscle, had increased on some capillary vessels in perimysium of GVHD muscle. ICAM-1 was not expressed on either muscle fibre itself or MHC class I^+ cells distributed throughout the endomysium and perimysium in GVHD muscle (data not shown).

Characterization of MHC class I^+ cells in GVHD muscle

We investigated whether all MHC class I^+ cells were capillary endothelial cells or not using double-immunofluorescence staining of HAM2 and HIS52. More than 500 cells were counted in 12 randomly-selected fields at x62.5 and percentages of $HIS52^+$ cells among MHC class I^+ cells were calculated. HIS52 stained 76% of MHC class I^+ cells (Fig. 2A,B).

We attempted to distinguish myosatellite cells from MHC class I^+ cells. All MHC class I^+ (endothelial and non-endothelial) cells in GVHD rat muscle were located outside the basement membrane detected by anti-laminin immunostaining (Fig. 3).

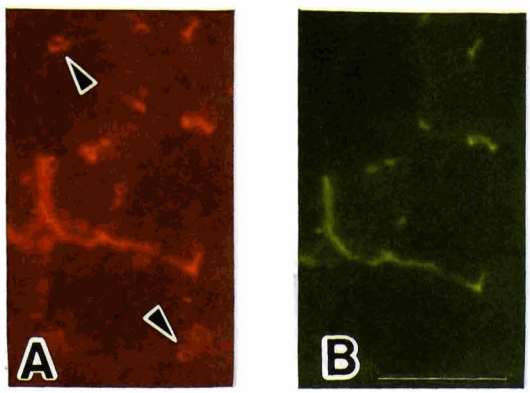

Fig. 2. Double immunofluorescence staining with HAM2 (MHC class I) **(A)** and HIS52 **(B)** in GVHD rat muscle. Seventy-six percent of MHC class I^+ cells are stained by HIS52, while 24% of MHC class I^+ cells are not stained by HIS52. Typical MHC class I^+ $HIS52^-$ cells are shown by arrowheads. Scale bar: 50 μm.

Discussion

Inflammatory reactions in muscle

In clinical reports of some polymyositis or dermatomyositis, MHC class I expression in muscle is usually associated with inflammatory cell infiltration (Arahata and Engel, 1984; Karpati et al., 1988; Emslie-Smith et al., 1989; Bartoccioni et al., 1994). Efforts to produce animal models of such inflammatory myopathies have been done in guinea pigs (Dawkins, 1965; Dawkins et al., 1971; Fulthorpe and Hudgson, 1975; Matsubara and Takamori, 1987), rats (Esiri and MacLennon, 1974) and mice (Tolnai, 1966; Hart et al., 1987; Rosenberg et al., 1987; Hohlfeld et al., 1988), but those experimental myopathies were not the complete reproductions and failed to elucidate pathogenesis of clinical diseases. These results may be taken as indicating low ability of immune response in muscle tissue.

According to the recent report about inflammatory myopathies by Bartoccioni et al. (1994), muscle fibres expressed ICAM-1 as well as MHC class I, and ICAM-1 was considered as an important adhesion molecule in pathogenesis of myopathies, stabilizing the interactions between T cells and their targets (Springer, 1990). Therefore, the expression of such molecules may be required for the success in producing animal models of myopathies involving immune phenomena. In the present study, muscle fibers did not express ICAM-I, while some of the capillary endothelial cells did.

A recent study about HLA expression and adhesion molecules on myoblasts and myotubes has shown that myoblasts constitutively express HLA class I (Hohlfeld and Engel, 1990) and a low level of lymphocyte function-associated (LFA) molecule 3 (LFA-3, CD58) (Goebels et al., 1992), and myotubes also constitutively express HLA class I but not LFA-3 (Hohlfeld and Engel, 1990). Interferon-γ (IFN-γ) induces myoblasts and myotubes to express HLA class I, class II and ICAM-I

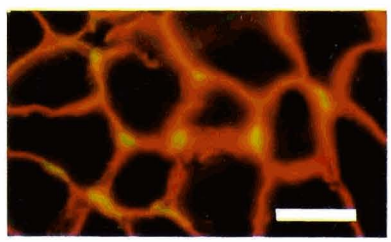

Fig. 3. Double-immunofluorescence staining with OX18 (MHC class I) (green, FITC) and anti-laminin antibodies (red, rhodamine) in GVHD rat muscle. MHC class I^+ cells (green spots) are apparently located outside the basement membrane (red, laminin stain). Scale bar: 25 μm,

(Bao et al., 1990; Hohlfeld and Engel, 1990; Mantegazza et al., 1991; Roy et al., 1991; Goebels et al., 1992). Tumor necrotizing factor-α (TNF-α) induces myoblasts to express HLA class II and ICAM-I (Goebels et al., 1992; Michaelis et al., 1993), and myotubes to express HLA class I, class II and ICAM-I (Michaelis et al., 1993). Thus, more factors such as TNF-α, IFN-γ or chemotactic factors which can induce myoblasts and myotubes to express histocompatibility antigens and cell adhesion molecules may be involved in inflammatory reaction in muscle tissue.

As a preliminary experiment, we injected allogeneic lymphocytes directly into GVHD rat muscle, where MHC class I was expressed on capillary endothelial cells and muscle fibres. However, those allogeneic lymphocytes did not remain in muscle, and disappeared quickly. Thus, allogeneic lymphocytes and MHC class I on muscle fibres or capillary endothelial cells in GVHD muscle tissues are evidently insufficient for inducing inflammatory reactions in muscle.

IFN-γ induces MHC class I expression on human myoblasts and myotubes (Bao et al., 1990; Roy et al., 1991; Kalovidouris, 1992; Michaelis et al., 1993). Subsequently, we preliminarily injected GVHD-rat serum into normal rats and confirmed that soluble factors may contribute to induce MHC class I expression in muscle. In this case, small fusiform cells in the perimysium and endomysium expressed MHC class I antigen, but muscle fibres did not. Inflammatory cell infiltration did not occur. Thus, MHC class I antigen expression in the present study may be induced by humoral factors such as IFN-γ (Bao et al., 1990; Hohlfeld and Engel, 1990; Mantegazza et al., 1991; Roy et al., 1991; Cifuentes-Díaz et al., 1992; Goebels et al., 1992; Kalovidouris, 1992; Hardiman et al., 1993; Michaelis et al., 1993) or other inflammatory factors carried by blood flow.

Unidentified cells in GVHD rat muscle

As mentioned previously, we observed MHC class I^+ small cells along the endomysium, which appeared to be either fusiform, round or sometimes dendritic. Among these small cells, 76% were identified as capillary endothelial cells by HIS52-positive staining. Other markers of endothelial cells, such OX43, OX2 and anti-von Willebrand factor VIII antibody were used to define endothelial cells as well, and the results showed that the proportion of endothelial cells among MHC class I^+ small cells did not exceed 76% (data not shown). Thus, the rest of them (24%) were not considered to be endothelial cells. Lack of staining by donor-specific MHC class I indicated that the MHC class I^+ unidentified cells originated from the host.

The rest of these MHC class I^+ cells were not myosatellite cells because these cells were located outside the basement membrane (Fig. 3). In addition, such cells did not carry MHC class II, CD4, CD5, CD8 and CD43 antigens, indicating that they may be neither granulocytes nor lymphocytes. The frequency of cells carrying macrophage-subset antigens was significantly low in normal muscle and only slightly increased, particularly along the perimysium, in GVHD muscle (Fig. 1D). Subsequently, the rest of the 24% of MHC class I^+ small cells could not be macrophages.

Consequently, it could be proposed that a certain type of MHC class I^+ cells, not belonging to endothelial cells, lymphocytes, granulocytes, macrophages or myosatellite cells, existed in GVHD rat muscle. It remains uncertain whether these unidentified cells exist in non-GVHD rat muscle, and whether they play an immunological role in muscle tissues, as endothelial cells do. These cells have not been previously reported in rat and human, and further investigation in rat and human muscle will be required. Morphologically, fibroblasts may be possible candidates for the unidentified non-endothelial MHC class I^+ cells which we describe in this manuscript. It is not unreasonable to consider that MHC class I antigen would be expressed strongly on fibroblasts under certain immunological conditions similar to salivary glands during GVHD (Hirozane et al., 1992), osteocytes (Shigetomi et al., 1993) and cardiac myocytes (Steinhoff, 1987) after allograft transplantation. More information on microstructures and histochemical aspects of the unidentified cells are necessary for precise characterization.

Acknowledgements. The authors thank Prof. P. Nieuwenhuis (Department of Histology and Cell-Biology, University of Groningen, Groniongen, Netherlands) for donation of HIS52, and Mr. M. Tamechika and Mr. A. Kumakura for excellent technical assistance.

References

Arahata K. and Engel A.G. (1984). Monoclonal antibody analysis of mononuclear cells in myopathies. I: Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T cells. Ann. Neurol. 16, 193-208.

Bao S., King N.J.C. and Dosremedios C.G. (1990). Elevated MHC class-I and class-II antigens in cultured human embryonic myoblasts following stimulation with γ-interferon. Immunol. Cell. Biol. 68, 235-242.

Barclay A.N. and Mason D.W. (1982). Induction of Ia antigen in rat epidermal cells and gut epithelium by immunological stimuli. J. Exp. Med. 156, 1665-1676.

Bartoccioni E., Gallucci S., Scuderi F., Ricci E., Servidei S., Broccolini A. and Tonali P. (1994). MHC class I, MHC class II and intercellular adhesion molecule-1 (ICAM-1) expression on inflammatory myopathies. Clin. Exp. Immunol. 95, 166-172.

Bland P.W. and Whiting C.V. (1992). Induction of MHC class II gene products in rat intestinal epithelium during graft-versus-host disease and effects of the immune function of the epithelium. Immunology 75, 366-371.

Brideau R.J., Carter P.B., McMaster W.R., Mason D.W. and Williams A.F. (1980). Two subsets of rat T lymphocytes defined with monoclonal antibodies. Eur. J. Immunol. 10, 609-615.

Cifuentes-Díaz C., Delaporte C., Datreaux B., Charron D. and Fardeau

M. (1992). Class II MHC antigens in normal skeletal muscle. Muscle Nerve 15, 295-302.

Dallman M.J., Thomas M.L. and Green J.R. (1984). MRC OX-19: A monoclonal antibody that labels rat T lymphocytes and augments in vitro proliferative responses. Eur. J. Immunol. 14, 260-267.

Dawkins R.L. (1965). Experimental myositis associated with hypersensitivity to muscle. J. Pathol. Bacteriol. 90, 619-625.

Dawkins R.L., Eghtedari A. and Holborow E.J. (1971). Antibodies to skeletal muscle demonstrated by immunfluorescence in experimental autoallergic myositis. Clin. Exp. Immunol. 9, 329-337.

Dijkstra C., Dopp E.A., Joling P. and Kraal G. (1985). The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 54, 589-599.

Duijvestijn A.M., van Goor H., Klatter F., Majoor G.D., van Bussel E. and van Breda Vriesman P.J.C. (1992). Antibodies defining rat endothelial cells: RECA-1, a pan-endothelial cell-specific monoclonal antibody. Lab. Invest. 66, 459-466.

Emslie-Smith A.M., Arahata K. and Engel A.G. (1989). Major histocompatibility complex class I antigen expression, immunolocalization of interferon subtypes, and T cell-mediated cytotoxicity in myopathies. Hum. Pathol. 20, 224-231.

Esiri M.M. and MacLennon I.C.M. (1974). Experimental myositis in rats: I. Histological and creatine phosphokinase changes and passive transfer to normal syngeneic rats. Clin. Exp. Immunol. 17, 139-150.

Fukumoto T., McMaster W.R. and Williams A.F. (1982). Mouse monoclonal antibodies against rat major histocompatibility antigens: two Ia antigens and expression of Ia and class I antigens in rat thymus. Eur. J. Immunol. 12, 237-243.

Fukumoto T., Kimura H., Naito M., Miyamoto M., Yamashita A. and Sugiyama H. (1984). Monoclonal antibodies to rat liver cell membrane glycoproteins. Mol. Immunol. 21, 285-291.

Fulthorpe J.J. and Hudgson P. (1975). Experimental allergic myositis. Neuropathol. Appl. Neurobiol. 1, 401.

Goebels N., Michaelis D., Wekerle H. and Hohlfeld R. (1992). Human myoblasts as antigen-presenting cells. J. Immunol. 149, 661-667.

Hardiman O., Faustman D., Li Z., Sklar R.M. and Brown R.H. (1993). Expression of major histocompatibility complex antigens in cultures of clonally derived human myoblasts. Neurology 43, 604-608.

Hart M.N., Linthicum D.S., Waldschmidt M.M., Tassell S.K., Schelper R.L. and Robinson R.A. (1987). Experimental autoimmune inflammatory myopathy. J. Neuropathol. Exp. Neurol. 46, 511-521.

Hickey W.F. and Kimura H. (1987). Graft-vs-host disease elicits expression of class I and class II histocompatibility antigens and the presence of scattered T lymphocytes in rat central nervous system. Proc. Natl. Acad. Sci. USA 84, 2082-2086.

Hirozane A., Fujikura Y., Sawada T., Shinozaki F. and Fukumoto T. (1992). Analysis of major histocompatibility complex (MHC) class I antigen in the rat salivary gland. Arch. Oral. Biol. 37, 147-151.

Hohlfeld R. and Engel A.G. (1990). Induction of HLA-DR expression on human myoblasts with interferon-γ. Am. J. Pathol. 136, 503-508.

Hohlfeld R. and Engel A.G. (1994). The immunobiology of muscle. Immunol. Today 15, 269-274.

Hohlfeld R, Müller W. and Toyka K.V. (1988). Necrotizing myopathy in SJL mice. Muscle Nerve 11, 184-185.

Jefferies W.A., Green J.R. and Williams A.F. (1985). Authentic T helper CD4 (W3/25) antigen on rat peritoneal macrophages. J. Exp. Med. 162, 117-127.

Kajiwara K., Hirozane A., Fukumoto T., Orita T., Nishizaki T., Kamiryo T. and Ito H. (1991). Major histocompatibility complex expression in brain of rats with graft-versus-host disease. J. Neuroimmunol. 32, 191-198.

Kalovidouris A.E. (1992). The role of cytokines in polymyositis: interferon-γ induces class II and enhances class I major histocompatibility complex antigen expression on cultured human muscle cells. J. Lab. Clin. Med. 120, 244-251.

Karpati G., Pouliot Y. and Carpenter S. (1988). Expression of immunoreactive major histocompatibility complex products in human skeletal muscles. Ann. Neurol. 23, 64-72.

Kimura H. and Willson D.B. (1984). Anti-idiotypic cytotoxic T cells in rats with graft-versus-host disease. Nature 308, 463-464.

Lampert I.A., Suitters A.J. and Chisholm P.M. (1982). Expression of Ia antigen on epidermal keratinocytes in graft-versus-host disease. Nature 293, 149-150.

Mantegazza R., Hughes S.M., Mitchell D., Travis M., Blau H.M. and Steinman L. (1991). Modulation of MHC class II antigen expression in human myoblasts after treatment with IFN-γ. Neurology 41, 1128-1132.

Mason D.W., Dallman M. and Barclay A.N. (1982). Graft-versus-host disease induces expression of Ia antigen in rat epidermal cells and gut epithelium. Nature 293, 150-151.

Matsubara S. and Takamori M. (1987). Experimental allergic myositis: ultrastructural, histochemical, immunological and immunohistochemical studies. Acta. Neuropathol. 74, 151-157.

McMaster W.R. and Williams A.F. (1979). Identification of Ia glycoproteins in rat thymus and purification from rat spleen. Eur. J. Immunol. 9, 426-433.

Michaelis D., Goebels N. and Hohlfeld R. (1993). Constitutive and cytokine-induced expression of HLA and cell adhesion molecules by human myotubes. Am. J. Pathol. 143, 1142-1149.

Rosenberg N.L., Ringel S.P. and Kotzin B.L. (1987). Experimental autoimmune myositis in SJL/J mice. Clin. Exp. Immunol. 68, 117-129.

Roy R., Dansereau G., Tremblay J.P., Belles-Isles M., Huard J., Labrecque C. and Bouchard J.P. (1991). Expression of major histocompatibility complex antigens on human myoblasts. Transplant. Proc. 23, 799-801.

Shigetomi M., Kawai S. and Fukumoto T. (1993). Studies of allotransplantation of bone using immunochemistry and radioimmunoassay in rats. Clin.Orthop. 291, 345-351.

Southwick P.L., Ernst L.A., Tauriello E.W., Parker S.R., Mujumdar R.B., Mujumdar S.R., Clever H.A. and Waggoner A.S. (1990). Cyanine dye labeling reagents-carboxymethylindocyanine succinimidyl esters. Cytometry 11, 418-430.

Springer T.A. (1990). Adhesion receptors of the immune system. Nature 346, 425-434.

Steinhoff G., Wonigeit K., Haverrich A., Schafers H.J. and Kemnitz J. (1987). Class I MHC antigen induction on myocytes in cardiac allograft rejection. Eur. Heart. J. 18 (Suppl. J.), 335.

Tamatani T. and Miyasaka M. (1990). Identification of monoclonal antibodies reactive with the rat homolog of ICAM-1 in the adherence of resting versus activated lymphocytes to high endothelial cells. Int. Immunol. 2, 165-171.

Tolnai G. (1966). Muscle lesions in mice injected with isologous and homologous muscle extracts. Nature 212, 1429-1431.

Williams A.F., Galfrè G. and Milstein C. (1977). Analysis of cell surface by xenogeneic myeloma-hybrid antibodies. Differentiation antigens of rat lymphocytes. Cell 12, 663-673.

Accepted August 25, 1995