Clin. exp. Immunol. (1986) **66,** 88–94

Aberrant expression of HLA-DR antigen on valvular fibroblasts from patients with active rheumatic carditis

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(Accepted for publication 7 April 1986)

SUMMARY

Immunofluorescence and immunoperoxidase staining was used to investigate the expression of Class II major histocompatibility antigens in myocardial tissue of 16 patients with acute rheumatic carditis. Aberrant expression of HLA-DR was examined using monoclonal anti-Ia antibodies and was detected on the valvular fibroblasts of those valves with ongoing active carditis. Sections of myocardial and valvular tissue from normal controls or from patients dying of other cardiac diseases did not express HLA-DR. The aberrant expression of HLA-DR on valvular fibroblasts could be important in triggering autoimmune destruction in that these cells could present self-antigens to sensitized T-lymphocytes which could initiate autoantibody production or direct destruction of local tissue.

Keywords MHC Class II antigens rheumatic heart disease

INTRODUCTION

Rheumatic heart disease is a disorder thought to be of immunological origin resulting from a previous Group A streptococcal pharyngeal infection (Cantanzaro *et al.*, 1954; McCarthy, 1981). Although the role of streptococcal infection in initiating the clinical and pathological processes of rheumatic fever is incompletely understood, the immunoglobulin levels and titres of antibody to *Streptococcus* exhibit an exaggerated and protracted rise 4 to 6 weeks after the start of the streptococcal infection (Kaplan, 1979). There is considerable evidence that the pathogenesis of rheumatic fever is a result of an abnormal host immune response either of the humoral or cellular type to the streptococcal antigens (Yang *et al.*, 1977). A variety of immunological abnormalities have been observed in this disease. These include the presence of circulating immune complexes and autoantibodies directed against cardiac, skeletal and smooth muscle (Kaplan *et al.*, 1984), heart valve fibroblasts (Kaplan, 1976) and neurons in the basal ganglia (Husby *et al.*, 1976). Although the proportion and percentage of circulating T cells are depressed in patients with acute rheumatic fever, examination of affected cardiac tissue shows significant interstitial lymphoid infiltrates containing a predominance of $OKT4^+$ helper T lymphocytes (Marboe *et al.*, 1985). It is likely therefore that cell-mediated immune mechanisms play an important role in the pathogenesis of

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acute rheumatic carditis. This idea has been substantiated by in-vitro studies which have demonstrated some cytotoxic activity on cardiac tissue of patient T cells activated by streptococcal antigens (Yang *et al.*, 1977; Senitzer & Freimer, 1984).

Class II major histocompatibility antigens (HLA-DR) play an important role in immunoregulation and antigen presentation (Benacerraf, 1978). The expression of these cell surface glycoproteins is normally restricted to B cells, activated T cells, macrophages, dendritic and some endothelial cells (Barclay & Mason, 1983). Recently Bottazzo, Pujol-Borrell & Hanafusa (1983) have suggested that local aberrant expression of HLA-DR by epithelial cells might enable antigens on them to stimulate T cells so as to initiate autoantibody production and direct destruction of local tissue or activation of effector B cells. In order to establish whether this mechanism was important in the pathogenesis of acute rheumatic carditis, we have examined myocardial tissue from these patients, employing monoclonal anti-Ia antibodies. These studies indicate that fibroblasts in the valvular tissue of patients with rheumatic carditis express Class II HLA-DR antigens.

METHODS AND MATERIALS

Patients

The 16 patients selected for this study all had clinically defined acute rheumatic carditis according to the modified Jones criteria (Special committee report Jones Criteria, 1958). These patients comprised seven males and nine females and ranged in age from 9 to 19 years. The clinical and histopathological data (see Table 1) showed typical features of acute rheumatic carditis and all valvular pathology was graded as III or IV according to the New York Heart Association criteria (Criteria Committee New York Heart Association, 1964). In addition, at surgery macroscopic evidence of active valvulitis was observed and histological examination revealed rheumatic activity in at least one of the valves in all patients.

Control tissue included portions of myocardial and valvular tissue obtained at necropsy from five apparently normal persons dying of traumatic deaths, eight patients dying of myocardial infarction, five patients dying of subacute bacterial endocarditis, three patients with non-infective merantic endocarditis and seven patients with valvular disease due to chronic rheumatic carditis.

Table 1. Clinical data on 16 patients with rheumatic carditis

Patient no.	Sex	Age	NYHA classification	Valve involved*	Presence of pyrexia	Sedimentation rate	C-reaction protein
1	M	11	III	MV	+	140	+
2	F	9	IV	MV, AV	+	150	+
3	F	12	III	MV, AV	+	85	+
4	M	10	III	MV	+	95	+
5	F	16	IV	AV	+	70	+
6	M	11	III	MV, AV	+	35	+
7	M	10	IV	MV, AV	+	46	+
8	F	16	III	MV, AV	+	70	−
9	F	14	IV	MV, AV	+	150	+
10	F	19	III	MV	ND	ND	−
11	F	11	III	MV, AV	−	6	ND
12	F	12	III	MV	−	32	+
13	M	10	III	MV	+	87	+
14	M	15	III	MV	+	40	+
15	F	16	III	MV, AV	+	40	+
16	M	9	IV	MV, AV	+	85	+

MV = Mitral valve; AV = Aortic valve; ND = Not done.

Other controls included active granulation tissue (peptic ulcer base) and in-vitro cultured skin fibroblasts.

Tissue obtained either at open heart surgery for valvular repair or replacement, or at autopsy, was snap frozen in liquid nitrogen and stored at −70°C until processed. Other portions of tissue were routinely fixed in formalin and embedded in paraffin. Frozen sections of mitral and aortic valve were studied in immuno-sandwich staining techniques employing both fluorescent and peroxidase conjugates. Paraffin embedded sections were stained using the immunoperoxidase technique or employing routine stains. Rheumatic activity was graded from acute to chronic according to the extent of inflammatory cells, oedema, fibroblastic proliferation, the degree of tissue vascularization and the presence of pathopneumonic phenomena such as Aschoff nodules and fibrinoid necrosis (Davies, 1980).

The monoclonal antibody OKIa (Ortho-mune, Ortho diagnostic systems Inc. New Jersey) directed against the constant region of the human DR portion of the Class II MHC antigen was employed in these studies. A second monoclonal antibody Anti-Ia NEI-011 (New England Nuclear, Boston, Massachusetts) was employed for confirmation.

Immunofluorescence staining

Frozen sections were first washed in PBS, pH 7·2, and were then incubated with the appropriate dilution of monoclonal anti Ia antibody for 30 min in a moist chamber. They were washed twice for 15 min in PBS, pH 7·2, and incubated with a purified fluorescent goat anti-mouse Fab antibody (Bionetics Laboratory Products, Litton Bionetics, Kensington, MD) for 30 min and then re-washed as above. Glycerol mounted sections were viewed under uv light (Polyvar, Reichert-Jung) and photographed at specific co-ordinates on the microscope slide. The coverslips were then removed and the sections washed and stained with haematoxylin-eosin. Slides were then repositioned on the microscope stage and photographed at the positions corresponding to the areas of fluorescence.

Immunoperoxidase staining

Frozen or paraffin embedded sections were stained with the peroxidase technique employing the Vectostain ABC kit PK 4002 (Vector Lab, Burlingame CA, USA). Briefly, slides were air dried, washed and the endogenous peroxidase activity quenched using a 0·3% solution of hydrogen peroxide in methanol for 30 min. After further washing the slides were incubated with normal goat serum for 20 min followed by a 30 min incubation with the monoclonal antibody appropriately diluted in PBS. Slides were then incubated for 7 min in a peroxidase substrate solution consisting of 0·1% diaminobenzadine tetrahydrochloride in PBS and 0·003% hydrogen peroxide solution in distilled water. Finally the sections were washed in tap water for 5 min and then counter-stained in Haematoxylin and clear mounted in DPX (viewed and photographed).

All sections were examined for positive staining in a double blind fashion by independent pathologists. The degree of positivity was graded from negative to ++++ according to the number of positively staining fibroblasts.

RESULTS

With the indirect fluorescent technique all valves which were histologically graded as having acute or sub-acute rheumatic activity showed numerous cells with positive bright green fluorescence under uv light (Fig. 1). When corresponding sections were stained with haematoxylin and eosin and the same field then viewed under the light microscope, the previously detected positively staining immunofluorescent cells could be identified as inflammatory cells and fibroblasts. Considerable numbers of positively staining histiocytes and occasional endothelial cells were usually identified and where Aschoff nodules were present they exhibited varying degrees of positive staining. There was no clear correlation between aberrant Ia expression on fibroblasts and the infiltration of mononuclear cells, as some valves with considerable aberrant expression showed minimal lymphocytic or histiocytic infiltration (Table 2). Valves which were classified as having early chronic rheumatic activity showed minimal fluorescence and similarly valves and myocardial tissue from

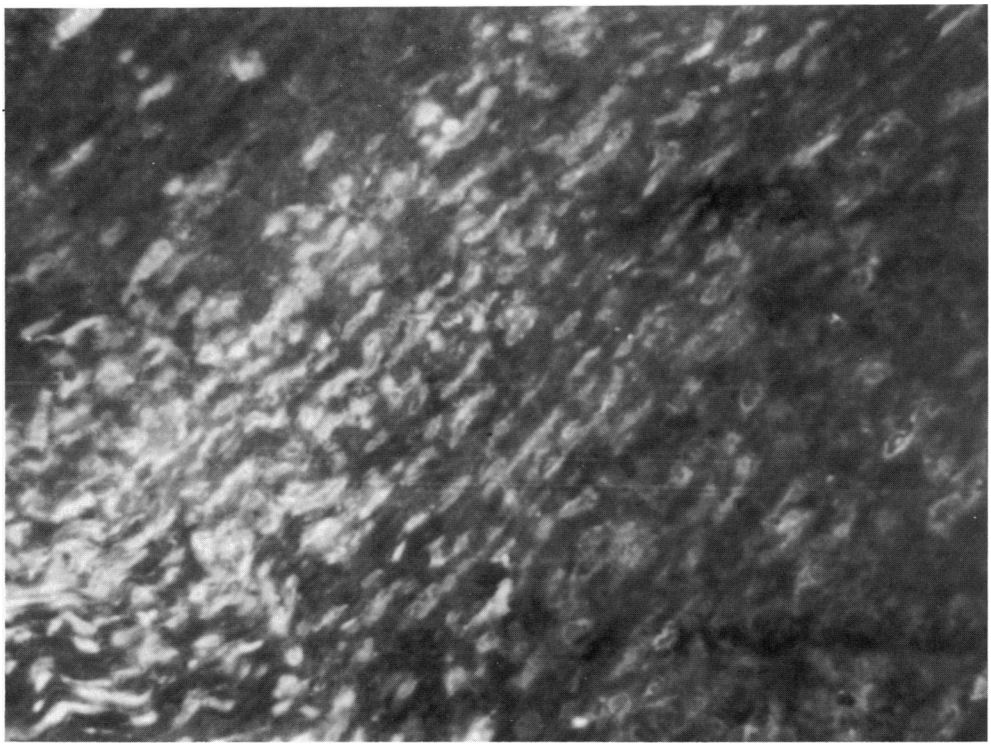

Fig. 1. Immunofluorescent staining of mitral valve from a patient with acute rheumatic carditis.

Table 2. Presence of HLA-DR expression on valve fibroblasts from mitral or aortic valves in patients with acute rheumatic carditis

Patient no.	Valves examined	Histopathological stage	MN cell infiltrate	Aberrant HLA-DR expressions
1	Mitral	Acute	+++	+++
2	Mitral	Acute	+++	++++
3	Mitral	Acute	++	+++
	Aortic	Acute	+++	++
4	Mitral	Acute	+	++
5	Aortic	Acute	++++	++++
6	Mitral	Subacute	++	++++
7	Aortic	Acute→Subacute	−	++++
	Mitral	Subacute	−	++
8	Mitral	Acute on chronic	+++	+++
	Aortic	Acute on chronic	++++	+++
9	Aortic	Subacute	++	++
	Mitral	Acute	++	+++
10	Mitral	Subacute→early chronic	++	++
11	Mitral	Subacute→early chronic	−	++
12	Mitral	Subacute→early chronic	+++	++++
13	Mitral	Subacute→early chronic	+++	++
14	Mitral	Early chronic	−	−
15	Mitral	Acute	++	+
	Aortic	Chronic	−	−
16	Mitral	Early chronic	−	+

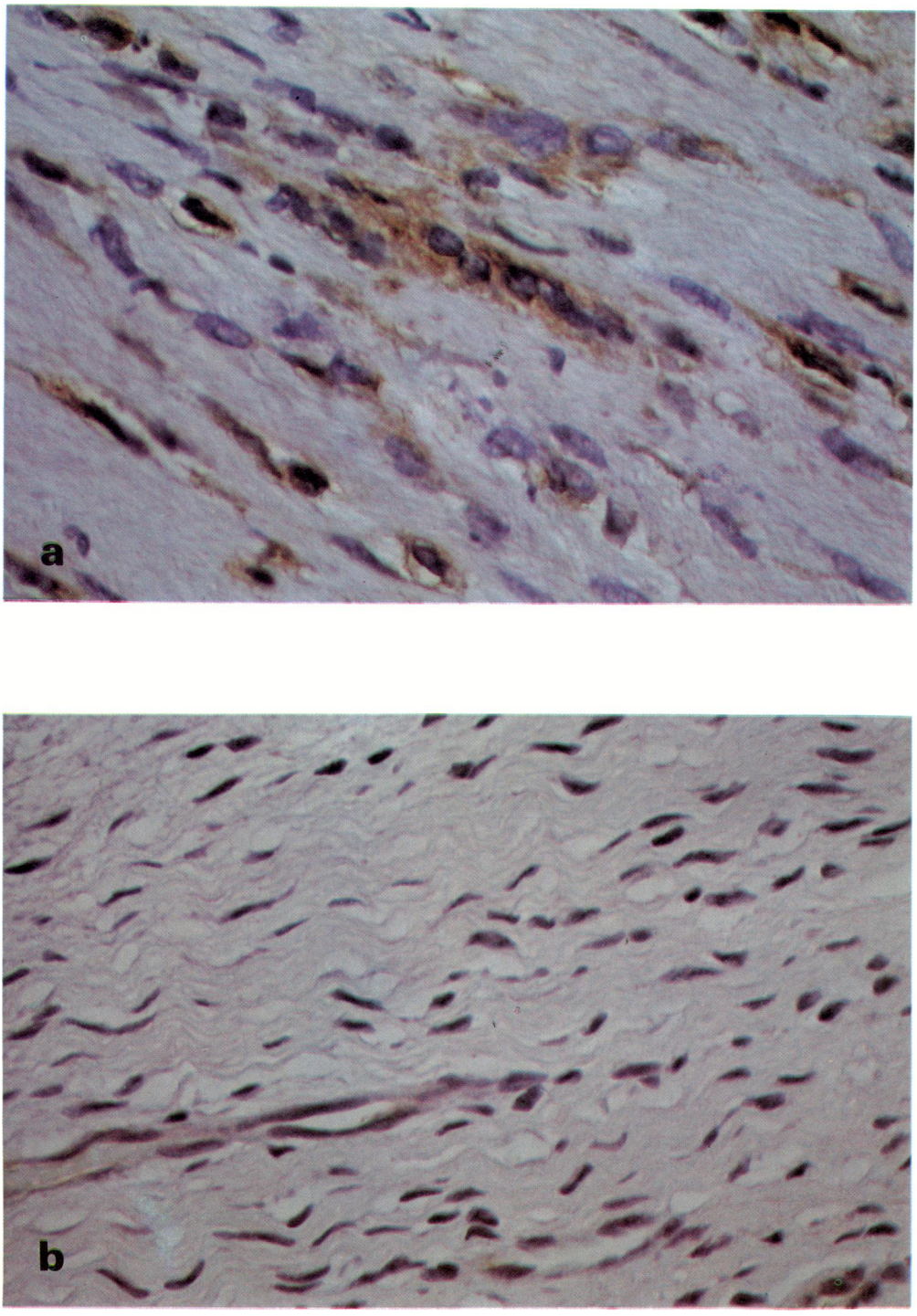

Fig. 2. Immunoperoxidase stained sections of mitral valves from (a) patient with acute rheumatic fever and (b) patient with myocardial infarction.

patients dying of traumatic deaths or from cardiac diseases other than acute rheumatic fever did not show positive fluorescence on fibroblasts. This included tissue from a group of patients with chronic rheumatic valvulitis. Fibroblasts from active granulation tissue or from long term fibroblast cultures stained negatively.

With the immunoperoxidase technique, positive brown granular staining could be easily identified on proliferating fibroblasts in valves showing acute or sub-acute rheumatic activity (Fig. 2). Once again valves classified as having chronic rheumatic activity showed few positively staining fibroblasts. Of particular interest was the observation that in some patients positive staining could be identified on one valve which showed positive histological rheumatic activity, whereas on another valve where the activity was chronic, staining for Ia was not observed (Table 2).

DISCUSSION

In the present study HLA-DR positive fibroblasts were observed in cardiac tissue from patients with acute rheumatic fever. Positivity was only observed on those valves which histologically showed acute activity, and chronically involved valves showed no positive staining with anti-Ia antibody. Fibroblasts expressing HLA-DR were not found in valves or myocardial tissue from normal individuals dying of traumatic deaths, or from patients dying of a variety of cardiac diseases including chronic rheumatic heart disease. This would suggest that these findings are restricted to, and important to, the pathogenesis of acute rheumatic carditis.

A question arose whether the positive Ia staining on fibroblasts represented a change which might exist in any acute inflammatory tissue. Fibroblasts, however, in acute granulation tissue at the base of acute peptic ulcers did not show this phenomenon, and fibroblasts taken from normal skin and cultured *in vitro* failed to express Ia antigens on their surface. Fibroblasts from the valves of acute rheumatic carditis patients stained positively employing both immunofluorescent and immunoperoxidase techniques and two different anti-Ia monoclonal antibodies gave similar results. Although infiltrating mononuclear cells also stained positively with anti-Ia antibodies, the positively staining fibroblasts could be easily differentiated on morphological grounds.

The Class II HLA-DR antigens are important in antigen presentation and T helper cells will only recognize antigens in the context of Class II molecules normally expressed only by a proportion of antigen-presenting cells (Benacerraf, 1978). It has been suggested that if cells are inappropriately stimulated to express Ia determinants, this might enable antigens on them to stimulate T cells to initiate an autoimmune attack (Hanafusa *et al.*, 1983; Jansson, Karlsson & Forsum, 1984). Such a mechanism has been suggested to explain the pathogenesis of Graves' disease and primary biliary cirrhosis (Balladini *et al.*, 1984). The aberrant expression of HLA-DR on fibroblasts in the valves of patients with acute rheumatic carditis may be important in the initiation of the development of heart lesions. Such a process could result in the triggering of an immune response against heart valve tissue and anti-heart antibodies have been observed in patients with acute rheumatic fever (Kaplan *et al.*, 1984). Although these antibodies have been shown to be non-cytotoxic (Kaplan *et al.*, 1984), considerable proliferation of fibroblasts has been observed in the heart valves of patients with acute rheumatic carditis.

It is possible that the HLA-DR antigens on the fibroblasts could be passively adsorbed from the considerable number of infiltrating immunocompetent cells which express DR antigens. In acute rheumatic fever the inflammatory infiltrate shows an abnormally high helper-suppressor T cell ratio (Marboe *et al.*, 1985). Although HLA-DR expression can be modulated by several factors including gamma interferon (Walker *et al.*, 1984), the cause of this phenomenon on cardiac fibroblasts in rheumatic carditis is unknown. Group A streptococci clearly play an important part in the aetiology of this disease, but a variety of viruses have also been implicated as possible secondary causative agents. The proposed viruses include coxsackie viruses and type 1,4 antigens of these viruses have been demonstrated within rheumatic myocardial tissue (Boonpacknavig, Udomsangpetch & Pongpanich, 1984). It is possible therefore that the virus together with the Group A *Streptococcus* may somehow be responsible for inducing the aberrant expression of HLA-DR on myocardial fibroblasts, perhaps through the effects of gamma interferon.

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