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MHC antigen expression in sequential biopsies from cardiac transplant patients—correlation with rejection

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SUMMARY

Class I induction on the myocardium of transplanted heart was investigated with regard to its temporal relationship to rejection episodes, how it is affected by anti-rejection therapy and whether it is dependent upon the presence of a T cell infiltrate in the biopsy. Sequential cardiac biopsies (total 114) from 11 patients from the time of transplant to 1 year after transplant were studied using immunocytochemical techniques. The effect of different immunosuppressive regimens on MHC antigen expression was also studied. All the biopsies diagnosed as showing rejection for the first time showed induction of Class 1 on the myocardium with 79% during subsequent rejection episodes. Class I induction was associated with a leucocyte infiltrate, not always containing T cells, and disappeared in 47% of biopsies taken 3–4 weeks after treatment with steroids and/or ATG. Increased expression of Class II, in particular DQ antigens on interstitial structures, paralleled Class 1 induction. MHC antigen expression returned to normal in 8/9 patients, at 1 year after transplant. Different immunosuppressive regimens affected the number of biopsies showing Class 1 induction on the myocardium. Our results suggest that in clinical heart transplantation class I induction is related to the rejection process.

Keywords Class I and Class II MHC antigens cardiac transplantation rejection heart

INTRODUCTION

Class I and Class II antigens are highly polymorphic glycoproteins encoded for by genes of the major histocompatibility complex (MHC) (Jongsma *et al.*, 1973). Class I antigens are important as targets for cytotoxic T cell responses, whereas Class II antigens stimulate immune responses by interaction with T helper cells (Benacerraf & Germain, 1978; Zingernagel & Doherty, 1979). Class II antigens have a relatively restricted tissue distribution, being found on B lymphocytes, dendritic cells, activated T cells, macrophages and some endothelia and epithelium (Natalie *et al.*, 1981; Fuggle *et al.*, 1983; Hirschberg, Moen & Thorsby, 1979). However they have been shown to be readily induced on tissues which normally do not express these antigens, such as kidney tubules and skin epithelium during rejection and graft-versus-host disease (Fuggle *et al.*, 1986; Lampert *et al.*, 1982).

Class I antigens were initially thought to be expressed on all nucleated cells (Berah, Hors & Dausset, 1970), though recent evidence suggests that they were not as ubiquitous as once thought (Daar *et al.*, 1984). Studies from this centre have demonstrated that myocardium from normal heart does not express Class I or Class II antigens although interstitial structures (endothelium and dendritic cells) express both these antigens. Aberrant expression of Class I on the myocardium was

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observed in 75% of cardiac biopsies from patients who had received cardiac transplantation (Rose *et al.*, 1986). Class II antigens were also more strongly expressed in these biopsies, though not on the myocardium but on infiltrating cells and interstitial structures. These original studies were unable to define the exact relationship between induction of MHC antigens during transplantation and rejection episodes. Here MHC antigen expression has been studied in sequential biopsies from 11 cardiac transplant patients in order to ascertain whether (1) aberrant MHC antigen expression is associated with rejection, (2) whether it is associated with the presence of a cellular infiltrate and whether the infiltrate contains T cells and (3) whether it returns to normal following treatment of rejection.

Recent studies have suggested that cyclosporine may inhibit induction of Class II MHC antigens (Groenewegen, Buurman & van der Linden, 1985). In order to ascertain whether different immunosuppressive regimens affect MHC antigen expression in the biopsies of heart transplant patients, a retrospective study was performed comparing biopsies from patients who had not received cyclosporine immunosuppression with biopsies from patients who had received cyclosporine in combination with either azathioprine and steroids or azathioprine alone.

MATERIALS AND METHODS

Sequential cardiac biopsies (total 114) from 11 patients were studied from the time of cardiac transplantation up to 3 months at weekly to two-weekly intervals, then at 6 months and 1 year. Nine patients underwent orthotopic and two patients underwent heterotopic transplantation. The age of the recipients varied from 8–59 years (mean $42 \pm 14{\cdot}4$). The primary disease in five patients was cardiomyopathy, in five patients ischaemic heart disease and in one patient rheumatic heart disease. The age of the donors varied from 8–40 years (mean $23{\cdot}8 \pm 11{\cdot}1$). All patients were receiving cyclosporine and azathioprine immunosuppression.

In order to study the effect of different immunosuppressive regimens, twenty-nine biopsies taken in the first 3 months after transplantation were studied. These biopsies were from three groups of patients receiving differing immunosuppressive regimens: Group I, nine biopsies from patients receiving azathioprine and corticosteroids; Group II, 10 biopsies from patients receiving azathioprine, corticosteroids and cyclosporine; Group III, 10 biopsies from patients receiving only azathioprine and cyclosporine.

Cardiac biopsies

Transvenous endomyocardial biopsies were performed routinely. Three pieces of right ventricular myocardium (2–3 mm^2) were fixed in formal saline, processed for paraffin wax embedding and used for routine histological assessment of rejection. One piece was snap-frozen and stored in liquid nitrogen until use. Frozen sections were stained using an immunoperoxidase technique as described previously (Rose *et al.*, 1986). Briefly, 6 μm sections were fixed in acetone and incubated with the appropriate dilution of monoclonal antibody (MoAb) (Table 1) for 30 min. After washing, the sections were incubated with a biotinylated goat anti-mouse IgG and IgM antibody (TAGO products, Tissue culture services), followed by a solution containing avidin-biotin-horseradish peroxidase complexes (Sera Labs). Immersion in diaminebenzadine tetrahydrochloride (0·3 mg/ml in PBS) and hydrogen peroxidase (0·1%) allowed visualization of peroxidase activity. The sections were counter-stained in Harris's haematoxylin.

Histology

Rejection episodes were diagnosed following histological assessment of the biopsy using the Billingham criteria. These criteria required the presence of an infiltrate containing pyroninophilic cells and/or oedema between the myocardial fibres (Billingham, 1981). Rejection episodes were treated with cortisteroids and/or anti-thymocyte globulin (ATG).

Immunoperoxidase staining

Table 1. Primary monoclonal antibodies used in immunoperoxidase staining of cardiac biopsies

Name	Specificity	Dilution	Reference/source
W6/32	Non-polymorphic Class I antigens	1/2	Barnstable *et al.* (1978)
B2N	β2-microglobulin	1/2	C. Navarrette & H. Festenstein, London Hospital.
CA2/ L227	Non-polymorphic Class II antigens	1/2	C. Navarrette & H. Festenstein, London Hospital.
HLA-DR	DR determinants of Class II antigen	1/10	Becton-Dickinson
Leu 10*	Polymorphic Class II antigens-DQ	1/40	Becton-Dickinson
Leu 4	All T cells	1/40	Becton-Dickinson
F10-894	Common leucocyte antigen	1/80	Dalchau, Kirkley & Fabre (1980)

* Identifies DQ in association with DR 1, 2, 4, 5, 6, 8, 9, w10.

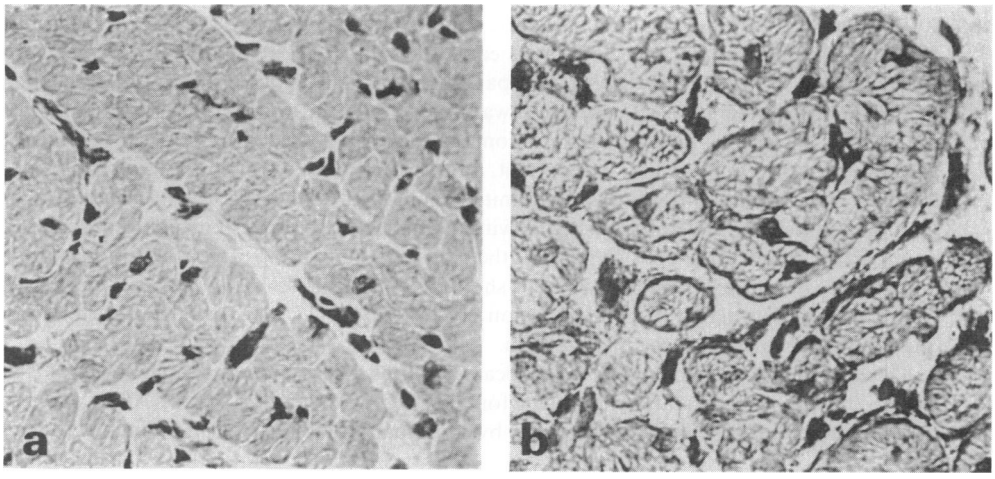

Fig. 1. (a) Class I (W6/32) antigen expression in normal donor heart taken at time of transplantation. Interstitial structures are positive but myocardium is negative; (b) Class I (W6/32) antigen expression in a heart biopsy showing rejection following cardiac transplantation. Myocardial membranes show positive staining. Patient receiving cyclosporine and azathioprine immunosuppression. Sections counterstained with haematoxylin. Photomicrograph × 320.

Class I. Slides stained with W6/32 were scored as positive or negative for expression of Class I on the myocardium.

Class II. Each biopsy was assessed for an increase in Class II positivity on interstitial structures and compared to normal staining ie. biopsy taken at time of transplantation.

T cells and leucocytes. Using a × 40 objective the number of positively stained cells were counted in 10 fields (one field corresponding to an area of 0·0625 mm^2) using an eyepiece graticule and the results expressed as the mean number of cells per 0·0625 mm^2 + standard deviation (s.d.).

Statistics. The significance of the results was assessed using the χ^2 distribution test and Student's *t*-test.

Table 2. Comparison of the numbers of leucocytes and T lymphocytes in normal biopsies and biopsies showing Class I expression on the myocardium (mean number ± s.d. per 0·0625 mm²)

	Normal heart taken at transplantation	Biopsies with normal Class I expression	Biopsies with Class I expression on myocardium
Leucocytes*	6·7 ± 3·6	7·5 ± 3·9	17·0 ± 7·2
T Lymphocytes†	0·2 ± 0·15	0·3 ± 0·54	4·5 ± 5·8

* $P = 0·001$; † $P = 0·001$ for comparison of normal biopsies with biopsies showing Class I induction on the myocardium.

RESULTS

Sequential study

Class I antigen

Association between Class I induction and rejection. Class I expression was not seen on the myocardium of the donor heart biopsy taken at time of transplantation. It was restricted to interstitial structures, which included small vessel endothelium and possibly dendritic cells (Fig. 1a).

Class I was induced on the myocardium as early as day 3 in one patient, 2–3 weeks in eight patients, and 6 and 10 weeks respectively in two patients after transplant (Fig 1b). Fifty-five percent (6/11) of the biopsies where Class I expression was first seen on the myocardium were treated for rejection. Of the five biopsies not showing rejection, two had been preceded and one was followed at 7 days by biopsies showing rejection. In contrast, only 31% (12/39) of the biopsies showing class I expression on the myocardium subsequent to initial expression were diagnostic of rejection.

One hundred percent (7/7) of biopsies showing first rejection episodes coincided with class I expression on the myocardium. In two patients the biopsies taken during the first rejection episode were too small to be analysed, but both patients showed Class I expression in biopsies taken 1 week and 2 weeks later. During subsequent rejection episodes 79% (11/14) coincided with biopsies showing Class I expression on the myocardium.

Class I expression, once induced on the myocardium, was shown to disappear in 47% (8/17) of the biopsies taken 3–4 weeks after treatment for rejection with steroids and or ATG. Patients showing persistent rejection were characterized by continual expression of Class I antigen in their biopsies.

Association between Class I induction and leucocyte infiltrate. Leucocytes were identified using MoAb F10-894. In biopsies showing normal Class I expression after transplant the number of leucocytes did not differ significantly from normal (Table 2). There was, however, a considerable scatter in the numbers (Fig. 2), with some biopsies showing a leucocyte infiltrate in the absence of Class I induction on the myocardium. Class I expression on the myocardium was associated with an increase in the leucocyte infiltrate which significantly differed from normal ($P = 0·001$) (Table 2). As with the biopsies showing normal expression of Class I, there was a considerable scatter in the numbers of leucocytes within the biopsies (Fig. 2).

Sixty-seven (29/43) of these biopsies with an infiltrate also contained significant numbers of T cells ($P = 0·001$) representing 38% of the total infiltrate (Table 2). Normal biopsies contained minimal to no T cells (Fig. 2).

Class II antigens

Expression of common determinants of Class II (L227/CA2) and DR (HLA-DR) antigens in normal donor heart was seen on the interstitial small vessel endothelium and dendritic cells (Fig.

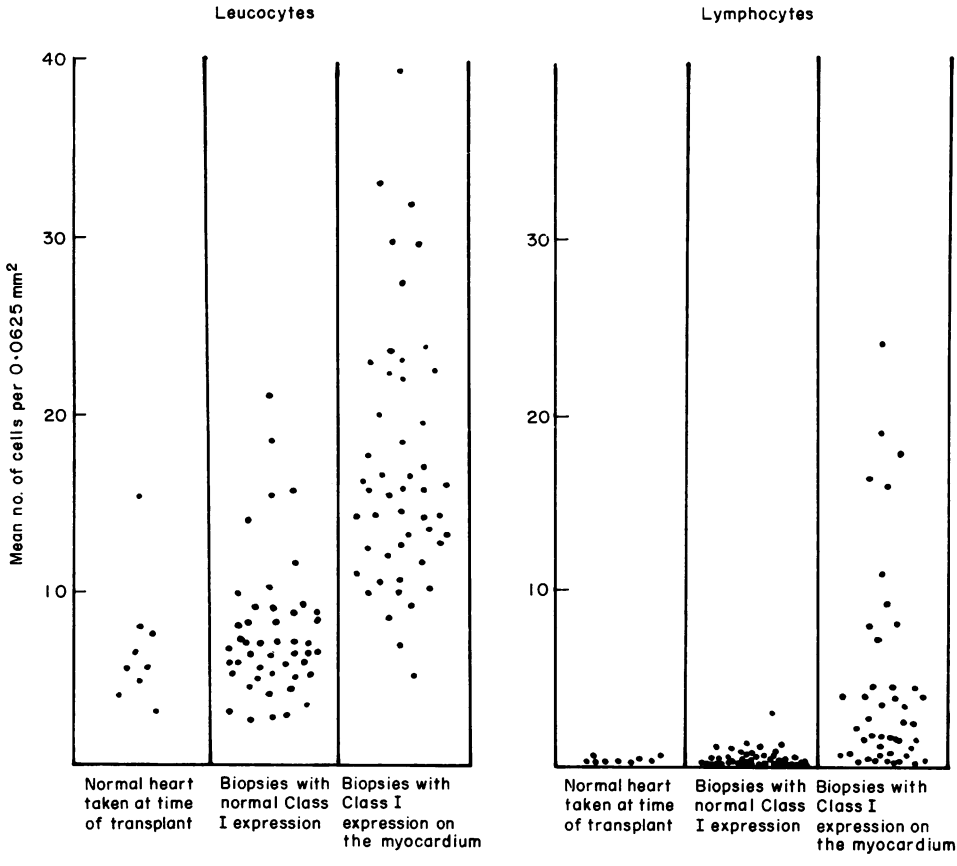

Fig. 2. Comparison of leucocyte and T lymphocyte numbers in biopsies taken at time of transplantation, biopsies with normal Class 1 expression and biopsies with Class I induction on the myocardium following transplantation.

3a). Seven patients received hearts from donors who were Leu 10^+ (Table 1). In these patients, DQ antigens (Leu 10) were normally absent or showed only weak staining of the interstitial structures (Fig. 3b). The myocardium was negative when stained with these antibodies.

Class II antigen expression was increased in all biopsies showing Class I antigen induction on the myocardium. The increase in staining was particularly striking with the DQ antigen (Fig. 3c). A large proportion of the increase in Class II antigen was seen on the cellular infiltrate, though it is possible that expression was also increased on the endothelium and discrete interstitial cells. The myocardium remained Class II negative.

Assessment at 6 and 12 months

Biopsies taken at 6 months showed that in 7/10 patients MHC antigen expression returned to normal, with no cellular infiltrate. The three patients showing continued expression of Class I on the myocardium were also diagnosed as rejecting in biopsies taken between 2 and 4 weeks previously. These biopsies showing Class I induction contained $10{\cdot}8 \pm 4{\cdot}4$ and $1{\cdot}2 \pm 1{\cdot}9$ leucocytes and T lymphocytes per 0·0625 mm² respectively.

At 1 year eight out of nine patients showed normal expression of MHC antigens in their biopsies. One patient showed focal expression of class I on the myocardium, having been treated for

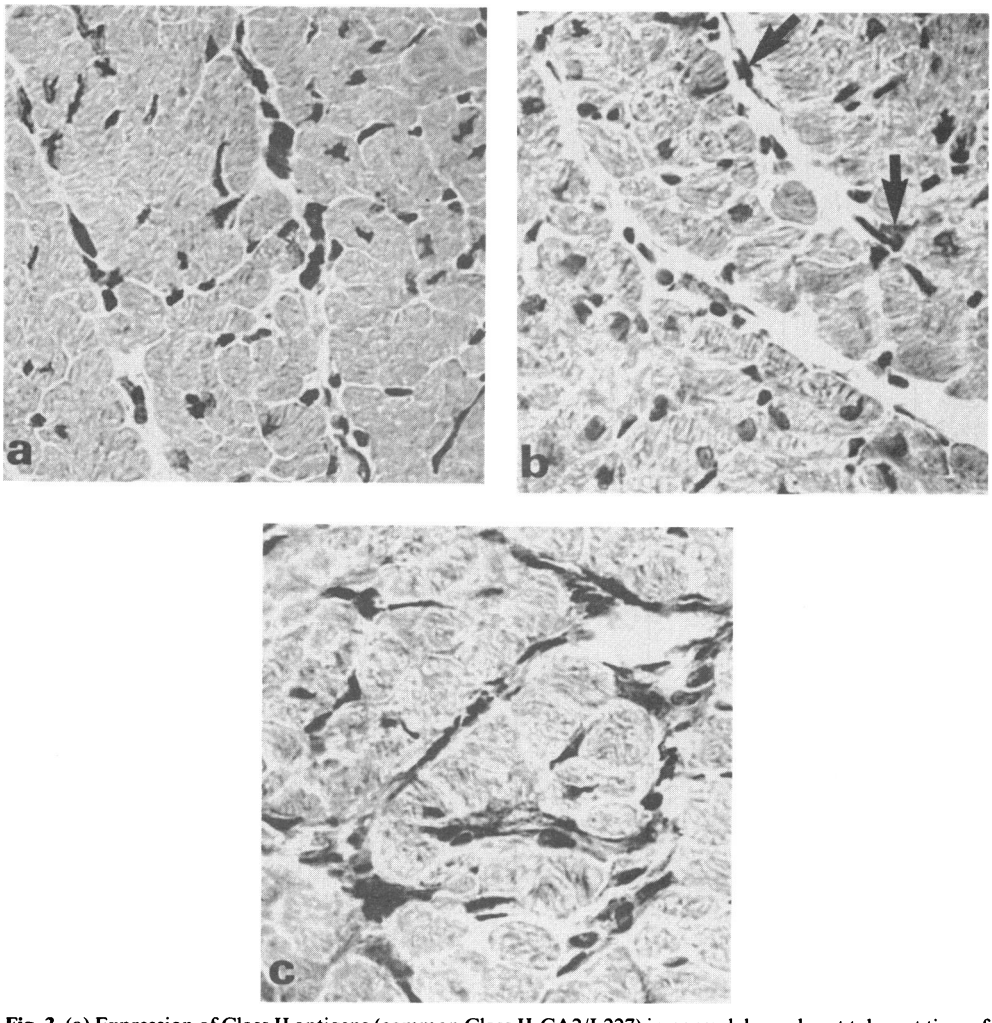

Fig. 3. (a) Expression of Class II antigens (common Class II-CA2/L227) in normal donor heart taken at time of transplantation. Interstitial structures are positive and myocardium is negative; (b) Expression of DQ antigens (Leu-10) in normal donor heart taken at time of transplantation. There is weak staining of some interstitial structures indicated by arrows; (c) Expression of DQ antigens (Leu-10) in a heart biopsy showing rejection following cardiac transplantation. Interstitial structures are strongly positive (compare to 3b). The myocardium remains Class II negative. Sections counterstained with haematoxylin. Photomicrograph × 320.

rejection 2 weeks previously. This biopsy contained 9·8 and 3·9 leucocytes and T lymphocytes per 0·0625 m^2 respectively. In two of the patients biopsies were not obtained at 1 year owing to technical difficulties.

Effect of immunosuppressive regimen

Induction of Class I antigen on the myocardium was seen in all three groups irrespective of immunosuppressive regimen (Table 3). Similarly $\beta 2$ microglobulin induction was found on the myocardium of these biopsies. Comparison of group 1 (azathioprine and steroids) with group II (cyclosporine, azathioprine and steroids) showed that the introduction of cyclosporine led to a decrease in percentage biopsies showing Class I induction ($P = 0{\cdot}05$). However comparison of group II with group III (cyclosporine and azathioprine) showed significantly larger numbers of biopsies in

Table 3. Comparison of the numbers of leucocytes and T lymphocytes in biopsies from patients on different immunosuppressive regimes (mean ± s.d)

	Biopsies with normal Class I expression	Biopsies with Class I induction on the myocardium	% biopsies showing Class I induction†
Group I			
F10-894	5·6 ± 5·2 ($n=3$)	16·9 ± 11·3 ($n=6$)	66%
Leu 4	1·0 ± 4·4 ($n=3$)	5·1 ± 3·5 ($n=5$)	
Group II			
F10-894	2·5 ± 2·2 ($n=8$)	21·6 ± 18·9 ($n=2$)	20%
Leu 4	0·2 ± 0·3 ($n=8$)	20·3 ± 22·1 ($n=2$)	
Group III			
F10-894	6·5 ± 3·5 ($n=2$)	20·3 ± 13·4 ($n=7$)	77%
Leu 4	0·9 ± 0·6 ($n=2$)	7·1 ± 9·6 ($n=8$)	

Group I, Azathioprine and steroids.
Group II, Cyclosporine, azathioprine and steroids.
Group III, Cyclosporine and azathioprine.
† $P=0·05$ comparing group I and II; $P=0·95$ comparing group I and III; $P=0·01$ comparing group II and III using χ^2 distribution test.

group III with Class I induction ($P=0·01$). It appeared therefore that patients taking triple therapy of cyclosporine, azathioprine and steroids had lower numbers of biopsies showing Class I induction than the other groups of patients. There was no difference between biopsies in Groups I and III.

As in the sequential study, the presence of Class I on the myocardium was associated with a leucocyte and T lymphocyte infiltrate (Table 3). However the number of biopsies investigated was small so statistical significance was not obtained.

Consistent with the sequential study Class II antigens were never found on the myocardium. However Class II antigen expression was increased on interstitial structures in biopsies showing Class I induction.

DISCUSSION

This study has confirmed our previous findings that normal myocardium does not express MHC antigens. However after transplants Class I antigens can be induced on the myocardium and the expression of Class II antigens on interstitial structures can be increased (Rose *et al.*, 1986). Studies in rat have also shown that MHC antigens are induced in the heart during rejection (Milton & Fabre, 1985). Here a prospective study of sequential biopsies was performed to understand the significance of MHC antigen induction following cardiac transplantation.

Although the first rejection episode was always associated with Class I induction, the correlation was not absolute with subsequent episodes. Thus, Class I expression on the myocardium was not necessarily diagnostic of acute rejection and could be found in the absence of histological signs of rejection, i.e. leucocyte infiltrate. We felt this was due to the fact that Class I expression, once induced, took 3–4 weeks to return to normal after anti-rejection therapy with steroids or ATG. A similar study has shown that Class II induction on kidney tubules after renal transplantation also persists for several weeks, although biopsies may be normal histologically (Fuggle *et al.*, 1986). The presence of a leucocyte infiltrate in biopsies showing no Class I induction on the myocardium is interesting. Evidence from this centre has shown that biopsies from patients receiving cyclosporine may contain large localized aggregates of cells in the myocardium and endocardium in the absence of any sign of damage to the myocardium (i.e. myocytolysis or oedema) (Rose & Yacoub, in press). These infiltrates do not contain pyroninophilic cells and the biopsies are not treated for rejection.

The biopsies from this study that contain an infiltrate in the absence of Class I expression on the myocardium correspond to these biopsies.

The induction of Class I and Class II antigens in the biopsies was associated with an increase in the leucocyte infiltrate, although not necessarily T cells. A study of renal transplant patients has also shown that induction of MHC Class II antigens in their biopsies is associated with a leucocyte infiltrate though with a significant T cell involvement (Fuggle *et al.*, 1986). Results from *in vitro* studies have shown that gamma-interferon (γ-IFN), a T cell derived lymphokine, is a powerful inducer of MHC antigens on a variety of cell types (reviewed by Pober *et al.* 1986). In addition, alpha and beta interferons produced by leucocytes and fibroblasts respectively have been shown to increase surface expression of Class I antigens but not Class II (Fellous *et al.*, 1979). Recently a mediator other than γ-IFN has been suggested as capable of inducing Class II expression (Groenewegen *et al.*, 1986). The observations presented here do not clarify whether MHC antigen induction precedes or follows myocardial damage. However recent evidence has shown that treatment of alloreactive T cell targets with γ-IFN greatly increases cytotoxicity (Blackman & Morris, 1985). Thus, it is tempting to speculate that the induction of Class I antigen on normally negative cardiac muscle cells by locally produced interferons leads to greater susceptibility of the cells to attack by cytotoxic T cells, and therefore may be an event preceding myocardial damage during rejection.

Class II antigens are necessary for the initiation of the immune response whether delayed type hypersensitivity or cytotoxic, so any increase in Class II antigen expression would be an important added stimulus to the reaction. It has been shown at this centre that endothelium expresses most of the Class II found in the heart (Rose & Yacoub, 1987). From *in vitro* studies endothelia have been shown to be capable of presenting antigen to T cells (Hirschberg, Bergh & Thorsby, 1980; Wagner, Vetto & Burger, 1985; Gibbs, Wood & Garovay, 1985), so it is reasonable to speculate that these cells may act as antigen-presenting cells and be an important stimulus of the rejection response in the heart. DQ antigens were not found in normal heart biopsies, but they increased dramatically following transplantation. These antigens have been found to be more susceptible to the effects of γ-IFN than either DR or DP (Capobianchi *et al.*, 1985).

It is known that cyclosporine inhibits the production of γ-IFN *in vitro* (Reem, Cook & Vilcek, 1983), and that injection of high doses of cyclosporine into normal dogs (Groenewegen *et al.*, 1985) and mice (Autenried & Halloran, 1985) leads to decreased expression of Class II antigen in skin, spleen and kidneys. Renal transplant patients on cyclosporine have been shown to have decreased Class II expression in their kidneys compared to patients on other forms of immunosuppressive regimes (Fuggle *et al.*, 1986). In this study a comparison of biopsies from patients on different immunosuppressive regimens revealed that there were differences in MHC expression in the heart biopsies following transplantation, but the differences were not caused solely by the presence of cyclosporine in the regimen. Biopsies from patients receiving triple therapy (cyclosporine, azathioprine and steroids) showed less Class I induction than patients on azathioprine and steroids or cyclosporine and azathioprine. These results are based on a small number biopsies and this study is currently being extended.

In conclusion the present study suggests that the presence of Class I antigen on the myocardium and DQ antigen on interstitial cells of transplanted hearts is related to rejection. Immunocytochemical identification of these antigens may be helpful in diagnosis of the first but not subsequent rejection episodes.

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