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Cell-mediated cytotoxicity in acute rat cardiac allograft rejection: an immunological and ultrastructural study

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Summary. To clarify the immune mechanism of cytotoxicity in acute cardiac allograft rejection, we observed interactions between cardiocytes and mononuclear cells using immunohistochemistry and light and electron microscopy. All allografted WKA rat hearts transplanted to F344 recipients stopped beating by the 7th day after the transplantation. The population of helper/inducer T cells (*Th*) and IL2R$^+$ cells was large for the first 3 days, whereas that of cytotoxic/suppressor T cells (Tc-s) and macrophages increased from the 4th day. The *Th*/Tc-s ratios were more than 2.0 until the 3rd day, then decreased to less than 1.0. In circulating T lymphocytes; the *Th*/Tc-s ratios were under 1.0 on the 1st, 6th and 7th days. Electron microscopically IL2R$^+$ cells, Tc-s and macrophages were often seen in close contact with the plasma membrane of the cardiocytes. The majority of IL2R$^+$ cells are NK cells, Tc-s and *Th*. Of these, the population of Tc-s was small until the 3rd day. Thus, NK cells play a pivotal role in the early stage of the rejection, and Tc-s and macrophages then aggravate cell-mediated cardiocyte injury.

Key words: Cell-mediated cardiocyte injury – Acute cardiac allograft rejection – Mononuclear cellular infiltration – Immunohistochemistry – Ultrastructure

Introduction

Mononuclear cellular infiltration is the most prominent histological feature in acute cardiac allograft rejection (Lowry et al. 1985). There has been considerable investigation of the composition and function of the infiltrating mononuclear cells (Christmas and MacPherson 1982; Ahmed-Ansari et al. 1988; Forbes et al. 1988); however, the precise mechanisms of the interactions between cardiocytes and infiltrating cells in cell-mediated cytotoxicity in the rejection remain unclear.

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With the use of cyclosporine, the effectiveness of rejection therapy has improved dramatically (Maugh 1980). However, immunosuppressive therapy makes the histological features of rejection more complex (Rose and Yacoub 1987). Rose et al. (1984) reported that biopsies from patients treated with cyclosporine contained large numbers of infiltrating leucocytes which were not involved in the rejection response.

We have reported the ultrastructural changes in the myocardium in acute cardiac allograft rejection and analysed cellular infiltration on the endocardial and epicardial sides of right and left ventricles (Hayashi et al. 1987). In the experiment reported here, we have focused on the cell-mediated cardiocyte injury, and observed the interactions between cardiocytes and infiltrating cells by light and electron microscopy. In addition, we analysed the subpopulations of circulating T lymphocytes in order to discover whether circulating T lymphocyte analysis reflects the immune repsonse *in situ*, and we have evaluated its clinical significance in the assessment of rejection. This ultrastructural and immunohistochemical study provides morphological evidence which may cast light on the nature of cell-mediated injury in acute cardiac rejection.

Materials and methods

Inbred rats of the strains WKA (RT1^k) and F344 (RT1^{lv1}) were used. Donor hearts of WKA males were grafted in recipient F344 males ($n=30$) by a method described elsewhere (Ono and Lindsey 1969). Two types of isografted rats were used as controls: F344 to F344 ($n=8$) for circulating T lymphocyte analysis, and WKA to WKA ($n=7$) for morphological study. The ischaemic time of the donor heart during transplantation was from 20 to 30 min. Graft rejection was monitored daily with electrocardiogram (ECG) and abdominal palpation. All animals were sacrificed at intervals of 24 h, or immediately if loss of ECG activity and/or loss of palpable contraction were noted, over 7 days after the transplantation. Two isografted control rats were observed for 60 days.

Before sacrifice, blood samples were taken for circulating T lymphocyte analysis. Analysis was carried out by the standard techniques of flow cytometry (Ellis et al. 1981) with monoclonal anti-

bodies: W3/13 (pan T cells), W3/25 (helper/inducer T cells: *Th*) or OX8 (cytotoxic/suppressor T cells: Tc-s) (Sera-Lab., Crawley Down, UK).

After blood sampling, donor hearts were perfused with periodate-lysine-paraform (PLP) solution (McLean and Nakane 1974) so as to wash out blood and preserve the antigen. The grafted hearts were then excised and tissue samples were obtained for study. The middle part of the left ventricle, which was not affected by operative trauma and thrombus, was fixed in 3% glutaraldehyde, post-fixed in 1% osmium tetroxide and embedded in epon. Ultrathin sections stained with uranyl acetate and lead citrate were examined under an electron microscope (Hitachi H-500 or H-800). Another part of the tissue specimen was fixed in 10% formalin and embedded in paraffin. The tissue sections were stained with haematoxylin and eosin (H & E) and Mallory-azan stains, and were examined by light microscopy.

For immunohistochemical studies the middle part of the left ventricle was once more fixed in PLP solution at 4° C for 4 h. It was then washed in phosphate-buffered saline (PBS) containing 10% sucrose and placed in a graded sucrose solution of PBS. It was washed again in PBS containing 20% sucrose and 10% glycerol. Then the tissue samples were placed in OCT compound (Tissue Tek II; Miles, Elkhart, Ind.). They were immediately snap-frozen in liquid nitrogen to obtain cryostat sections of 4–6 μm thickness.

These sections were incubated with monoclonal antibody (mAb): W3/13 (pan T cells), CD4 (helper/inducer T cells: *Th*), CD8 (cytotoxic/suppressor T cells: Tc-s), OX-39 (interleukin 2 receptor positive cells: $IL2R^+$), or with OX41 (monocyte/macrophage: Mø) (Chemicon International, El Segundo, Calif.) as a first stage reagent. After additional washing with PBS, the sections were incubated with goat anti-mouse IgG (AFFI-PURIF) (Chemicon International) as the second stage reagent.

For immunolight microscopy, the sections were treated with diaminobenzine (DAB)-peroxidase solution, dehydrated and counterstained with Mayer's haematoxylin. In the sections, cells stained positive with mAb, W3/13, CD4, CD8, IL2R or Mø were identified under the light microscope by red-brown reaction products on the surface or within the cytoplasm.

For immunoelectron microscopy, the incubated sections were post-fixed in 2% glutaraldehyde, allowed to react with DAB peroxidase, and again fixed with osmium tetroxide. They were then dehydrated in graded ethanols and embedded in luveac. Ultrathin sections were examined without additional staining, with an electron microscope. In the sections stained with each mAb, positive lymphocytes were identified by the DAB reaction product, which was usually present only focally along the membranes of the cell surface and cytoplasm. Occasionally, the reaction product was uniformly distributed along the entire cell membrane.

As a positive control in the immunoperoxidase procedure, spleen tissue of the recipient, fixed in PLP, was employed and was treated in the same way as above. As a negative control, some sections of donor hearts were first incubated with mouse ascitic fluid, which was obtained from animals injected with non-secreting hybridoma cells, and then treated with the second-stage reagent as above.

Quantitative measurement was made as follows:

1. Infiltrating mononuclear cells were counted in sections of the tissue stained with H & E under a light microscope. Fields of view corresponding to 0.2 mm^2 (sufficient to observe the morphological changes in the middle part of the left ventricle in rat hearts) were randomly selected, and the numbers of mononuclear cells were counted using the ×1000 objective.
2. Sections stained with each mAb were examined under the microscope, using the ×1000 objective. The percentage of infiltrating cells was measured by making a random examination of 500 mononuclear cells (MNCs). Total numbers of positive cells with each mAb were calculated as follows:

Total number = percentage of cells binding with each mAb × total number of MNCs per 0.2 mm^2.

All results obtained by the enumeration were expressed as mean ± standard deviation (SD). Statistical analysis was performed using the Kruskal-Wallis rank test and the Spearman rank test, and P values of less than 0.05 were considered significant.

Results

In all heart allografts, reduction of ECG voltage and loss of palpable contraction were noted by the 7th day after the transplantation (mean 5.8 ± 1.0 days after the transplants). In contrast, contraction was clearly palpable in all isografted hearts even 60 days later.

The population of *Th* tended to increase on the 4th day, whereas that of Tc-s tended to increase on the 1st and 7th day and decrease on the 4th day (Table 1). The ratio of *Th* to Tc-s changed significantly after the transplantation ($\chi o^2 = 16.30$), and tended to decrease with the passage of time ($r_s = -0.179$). It was under 1.0 on the 6th and 7th day after the transplantation.

Macroscopically, heart allografts were often dilated, and had haemorrhagic-looking, swollen, mottled sites and pale areas of myocardial necrosis. The left ventricles often contained abundant stasis thrombi.

On light microscopy the isografted control rats showed almost normal morphological configuration and the myocardium, and cellular infiltration was absent throughout the observation period. Sixty days after the transplant, however, disarrangement and atrophy of the myocardial cells and a moderate degree of collagen fibre proliferation were seen in the subepicardial region. These morphological changes are probably a form of atrophy resulting from inactivity of the grafted hearts; in this experiment operative ischaemia (Smith et al. 1987) is thought to have been minimal.

The earliest histological manifestations of acute rejection were diffuse interstitital oedema around the intramural vessels and focal mononuclear cellular infiltration of varying intensity in the interstitium. The area of infiltration extended rapidly over the myocardium, and the cellular infiltration was followed by various degrees of myocardial degeneration and necrosis (Fig. 1).

The MNCs in 0.2 mm^2 were counted, and the percentage of cells binding with each mAb was obtained (Table 2). MNCs changed significantly after the transplantation and continued to increase ($\chi o^2 = 27.83$, $P < 0.001$; $r_s = 0.918$, $P < 0.001$). The population of *Th* and $IL2R^+$ cells was large for the first 3 days, whereas that of Tc-s and Mø increased from the 4th day after the transplantation. The ratio of *Th* to Tc-s showed values from 2.21 to 2.31 for the first 3 days, which decreased to 0.89 ± 0.10 on the 4th day and remained under 1.0 thereafter. All the calculated numbers of $W3/13^+$, $CD4^+$, $CD8^+$, $IL2R^+$ cells, and Mø in 0.2 mm^2 increased constantly throughout the observation period, affected by the increase of MNCs.

From the day after the transplantation, many lymphocytes were observed in the interstitium on electron microscopy, making close contact with Mø or with other lymphocytes. Some of these lymphocytes were identified as *Th* by immunoelectron microscopy (Fig. 2). During the first 3 or 4 days, *Th* and $IL2R^+$ cells were prominent in number, and then the number of Tc-s and Mø increased.

Table 1. Circulating T lymphocyte analysis

		Days	0	1	2	3	4	6	7	χo^2
W3/13$^+$	(%)	Mean	67.2	66.1	62.2	66.6	67.8	60.1	47.9	8.97
		SD	4.2	22.5	8.8	6.1	5.3	0.4	3.2	
W3/25$^+$	(%)	Mean	38.9	30.9	34.4	36.2	41.8	31.9	30.1	11.58
		SD	1.7	7.8	5.9	7.1	3.5	1.7	3.5	
OX8$^+$	(%)	Mean	33.9	41.0	33.0	33.5	28.2	36.4	43.2	11.97
		SD	1.2	18.2	0.2	3.0	2.9	4.5	4.1	
T4/T8		Mean	1.14	0.79	1.04	1.10	1.48	0.87	0.70	16.30*
		SD	0.07	0.16	0.18	0.29	0.08	0.06	0.14	

* $P<0.05$

Some of the lymphocytes in the interstitium were adjacent to myocardial cells and occasionally protruded a part of the cytoplasm, apparently making contact with the myocardial cells via pseudopodia (Fig. 3). IL2R$^+$ cells and some other lymphocytes were seen beneath the basement membrane of some cardiocytes, making close contact with the plasma membrane of the cardiocyte (Fig. 4). Beneath the plasma membrane of the contact area disorganized mitochondria were often observed.

IL2R$^+$ cells, Tc-s, and Mø were often seen in the widened intercellular spaces of dissociated intercalated discs (Fig. 5), where hemi-desmosomes were occasionally observed (Fig. 6). It appears that these cells had breached the basement membrane of the cardiocytes, and made their way into the space within the intercalated disc. Such cells were also seen in the widened intracytoplasmic confines of some cardiocytes. It seems that they had invaded from the lateral side of the cardiocyte and approached near to the centre of the cardiocyte (Fig. 7).

Most of the myocardial cells showed an almost normal morphological appearance in the very early stage of the rejection, whereas a large number of cardiocytes underwent degenerative changes within 1 week after the transplantation. The damaged myocardium sites appeared to increase in area in parallel to the increase of cellular infiltration. IL2R$^+$ cells and Tc-s were often in close contact with morphologically normal cardiocytes; however, Mø were often in contact with degenerated cardiocytes. These cells were sometimes seen to have invaded necrotic myocardial cells in the vicinity of intercalated discs. The necrosis often involved one of two adjacent cells which were joined to each other by an intercalated disc (Fig. 8).

Other changes in the cardiocytes were general loss of myofilaments, disruption of the intercalated discs, and amorphous, moderately electron-dense inclusions in the mitochondria (Fig. 9). These changes were seen at a late stage of the observation period.

From 3 to 4 days after the transplantation, morphological alterations in capillaries were often observed, consisting of endothelial cellular swelling, an increase of pinocytotic vesicles, and irregularity of the luminal surface (Fig. 10). On several occasions, some lymphocytes were seen which appear to have penetrated the capillary wall.

Discussion

The most important mechanism involved in the rejection of an allograft is cell mediated (Cramer 1987). Initiation of the cell-mediated immune reactions requires antigen-presenting cells such as macrophages or dendritic cells. Initially, the antigen-presenting cells activate the small number of helper T lymphocytes. Activated helper T cells elaborate lymphokines, some of which activate macrophages and also recruit other lymphocytes and monocytes-macrophages to participate in the rejection (Herscowitz 1985).

In this study, many lymphocytes were seen from the 1st day after the transplantation. Of these, the population of *Th* and IL2R$^+$ cells was large for the first 3 days, whereas that of Tc-s and Mø increased from the 4th day. *Th* were often in close contact with Mø or with other lymphocytes in the interstitium. This close contact may be the morphological representation of cellular interaction in the initiation of cell-mediated cardiocyte injury. Thus, *Th* play an important role along with Mø in providing positive signals for a number of cells that are involved in the expression of cell-mediated immune reactions.

Activated lymphocytes or Mø bind to target cells to inflict damage. Some lymphocytes in the interstitium were adjacent to cardiocytes and protruded a part of their cytoplasm. It might be that these lymphocytes were on the way to binding to the cardiocytes as target cells.

It is generally accepted that there are four effector cells which inflict damage on target cells through direct contact with them: (1) cytotoxic T cells (Tc-s), (2) K cells (ADCC), (3) NK cells, and (4) macrophages (Bellanti and Rocklin 1985). We observed many IL2R$^+$ cells during the first 3 or 4 days, and many Tc-s and Mø after the 4th day. These effector cells had come into close contact with the plasma membrane of cardiocytes. It is apparently at these contact points that the cells exert their cytotoxicity on the myocardial cells. IL2R$^+$ cells consist chiefly of NK cells, Tc-s and *Th*. Of these, the population of Tc-s was small for the first 3 days. Therefore, most of these IL2R$^+$ cells seen in close contact with plasma membrane of cardiocytes in the early stage of the rejection appear to be compatible with NK cells. Recently, much attention has been paid to the role

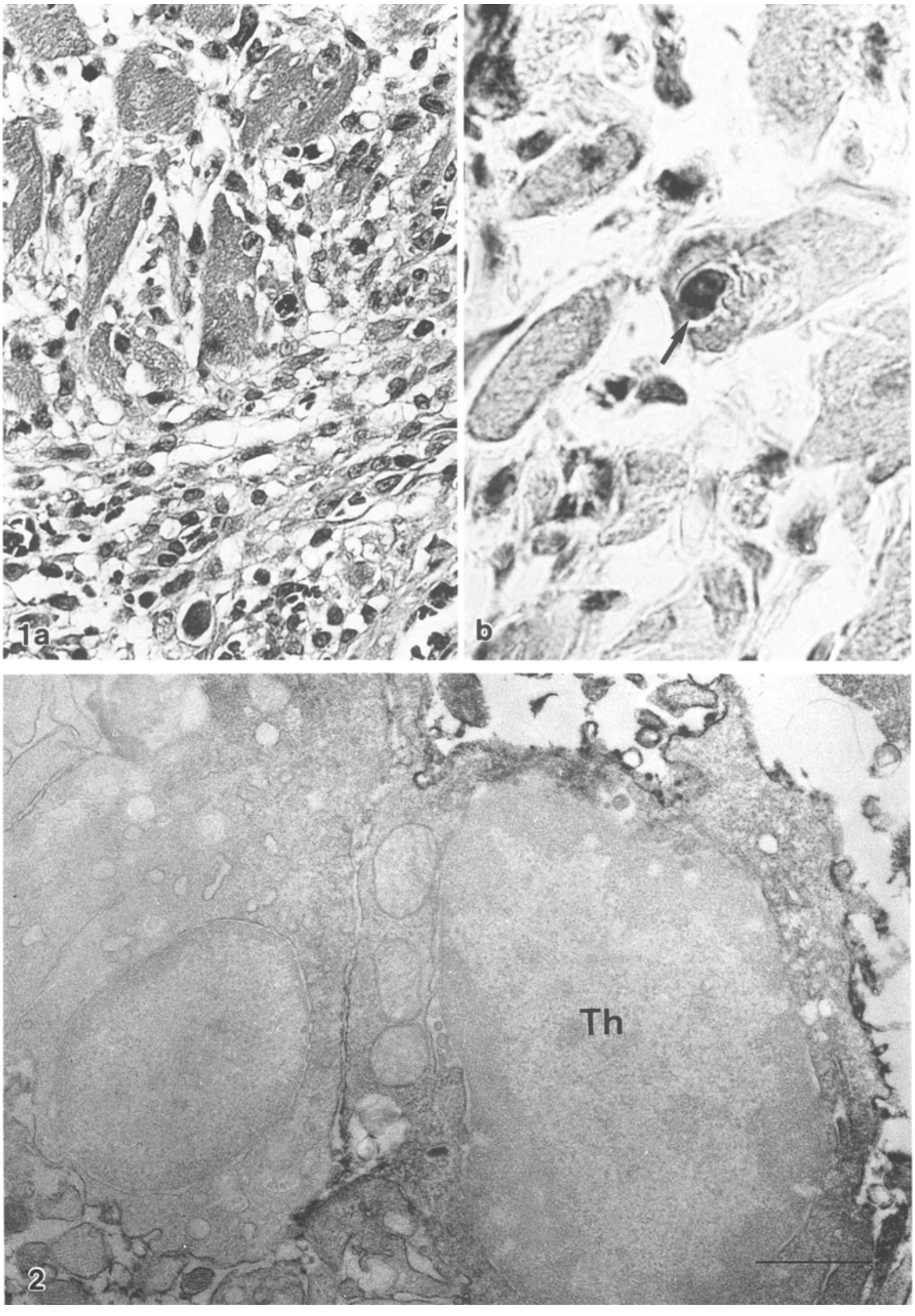

Fig. 1. **a** Light micrograph of the allografted heart on the 4th day after the transplantation. Very numerous mononuclear cells are present in the myocardium. H & E. **b** Light micrograph of the myocardium stained with CD8 monoclonal antibody on the 4th day. One of the $CD8^+$ cells is seen near the centre of a cardiocyte (*arrow*)

Fig. 2. Immunoelectron micrograph of $CD4^+$ cell (*Th*) in the interstitium of the allografted heart. The $CD4^+$ cell is in close contact with another lymphocyte. *Scale bar*, 1 μm

Table 2. Number of mononuclear cells (MNCs) in 0.2 mm^2, percentage of cells binding monoclonal antibody per unit, and ratio of T4 to T8

		Days	0	1	2	3	4	5	6	7	r_s
MNCs		Mean	6.0	12.0	43.2	58.6	101.3	114.3	143.3	152.0	0.918**
		SD	2.0	2.2	15.8	20.6	20.0	48.7	25.5	23.3	
W3/13$^+$	(%)	Mean	47.4	49.0	48.8	50.3	39.0	41.4	42.9	41.0	−0.435*
		SD	3.3	1.8	15.7	16.3	2.9	3.0	5.4	1.5	
CD4$^+$	(%)	Mean	31.9	33.8	33.6	38.5	25.0	18.5	22.5	19.0	−0.720**
		SD	1.7	3.3	4.9	4.8	5.0	5.6	2.5	3.1	
CD8$^+$	(%)	Mean	14.1	14.7	15.3	18.1	27.9	23.8	33.6	27.8	0.796**
		SD	1.5	1.8	3.0	5.0	3.3	5.8	2.3	1.4	
IL2R$^+$	(%)	Mean	32.4	35.9	30.0	32.1	30.8	28.9	34.2	29.6	−0.279
		SD	0.8	2.1	5.1	5.8	1.2	5.9	2.8	1.4	
Mø$^+$	(%)	Mean	10.1	13.3	13.7	19.1	28.1	28.8	33.5	36.0	0.911**
		SD	0.3	2.9	1.2	4.7	1.5	7.8	6.3	3.9	
T4/T8		Mean	2.27	2.31	2.23	2.21	0.89	0.79	0.67	0.68	−0.836**
		SD	0.14	0.25	0.26	0.42	0.10	0.20	0.06	0.10	

* $P<0.05$, ** $P<0.001$

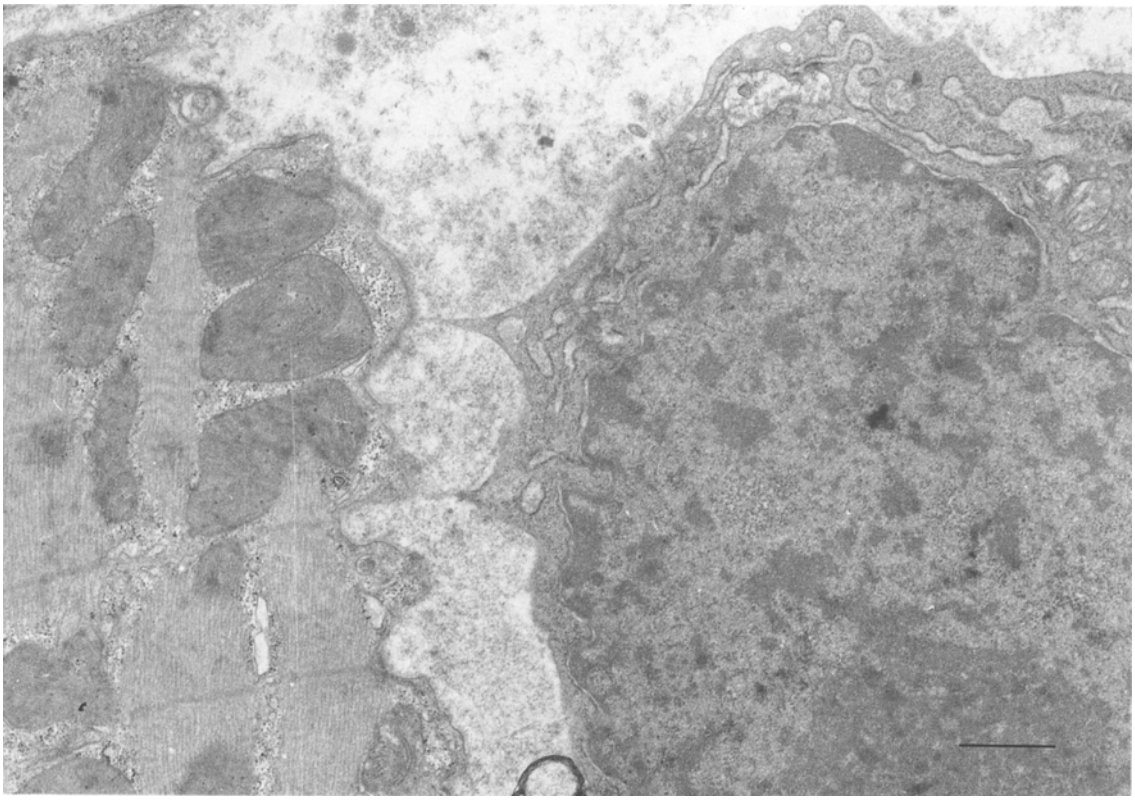

Fig. 3. Electron micrograph of a lymphocyte which has protruded a part of cytoplasm making contact via pseudopodia with a cardiocyte. *Scale bar*, 1 μm

of NK cells in the rejection response (Marboe et al. 1983). Our data suggest that NK cells play an important role in the early stage of the rejection, and that Tc-s and Mø then aggravate cell-mediated injury.

IL2R$^+$ cells, Tc-s, and Mø were often seen in widened intercellular spaces of dissociated intercalated discs, where hemi-desmosomes were occasionally observed. It appears that these cells had breached the basement membrane of the cardiocyte and made their way into the space within the intercalated disc. Such cells

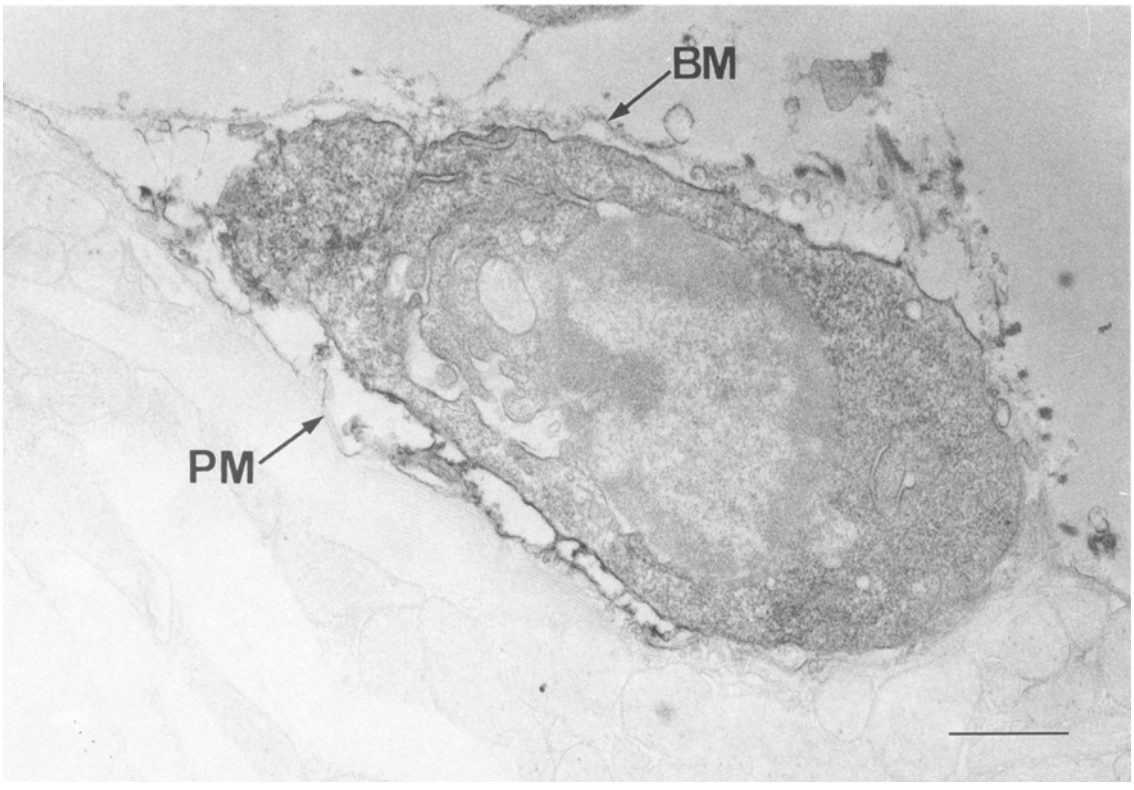

Fig. 4. Immunoelectron micrograph of IL2R$^+$ cell in a space between basement membrane (*BM*) and plasma membrane (*PM*) of a cardiocyte. *Scale bar*, 1 μm

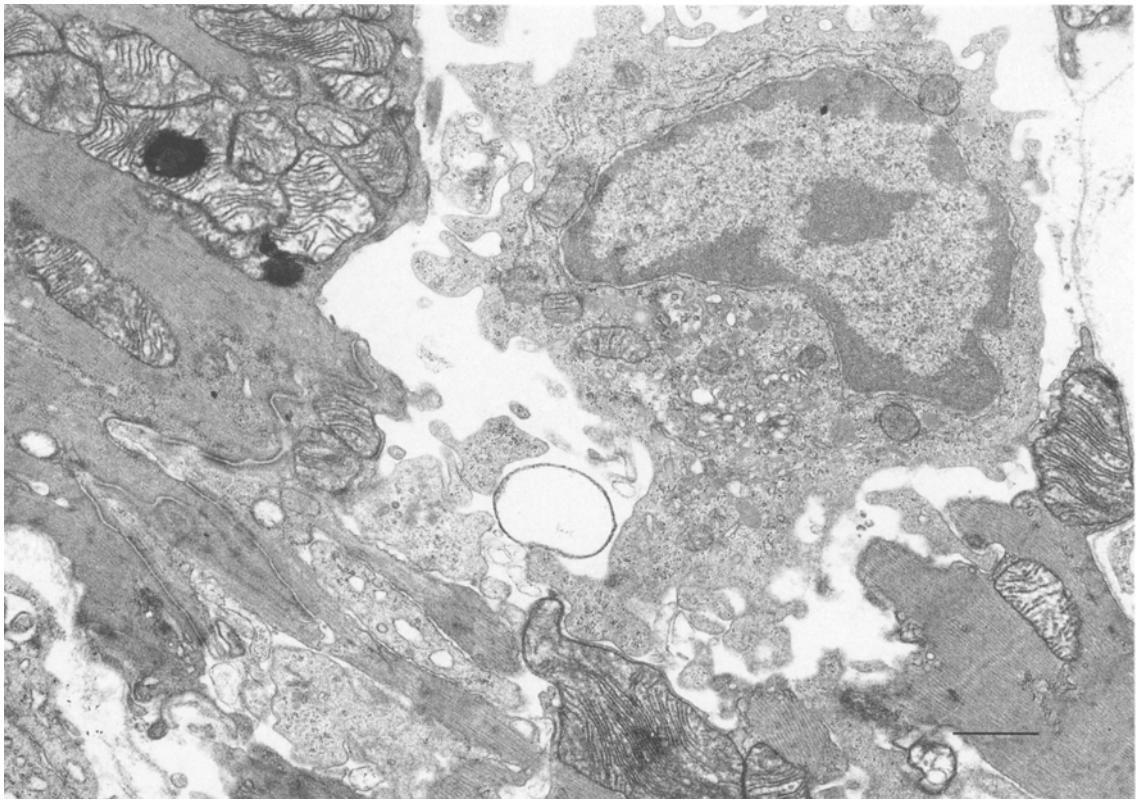

Fig. 5. Electron micrograph of the myocardium in an allograft. A large granular lymphocyte is in a large space between basement membrane and plasma membrane of a cardiocyte, making close contact with the cardiocyte at several places. *Scale bar*, 1 μm

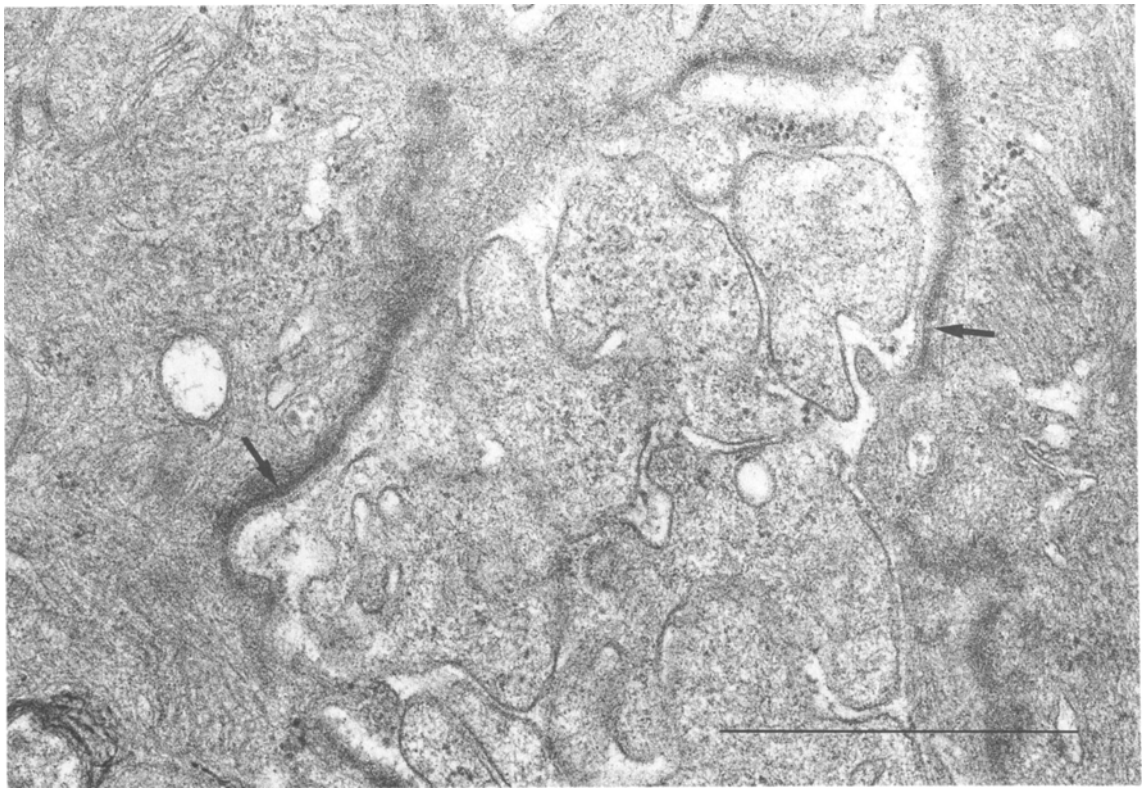

Fig. 6. Electron micrograph of the myocardium in an allograft. Along dissociated intercalated disc there are occasionally hemi-desmosomes of intercalated disc (*arrows*). *Scale bar*, 1 μm

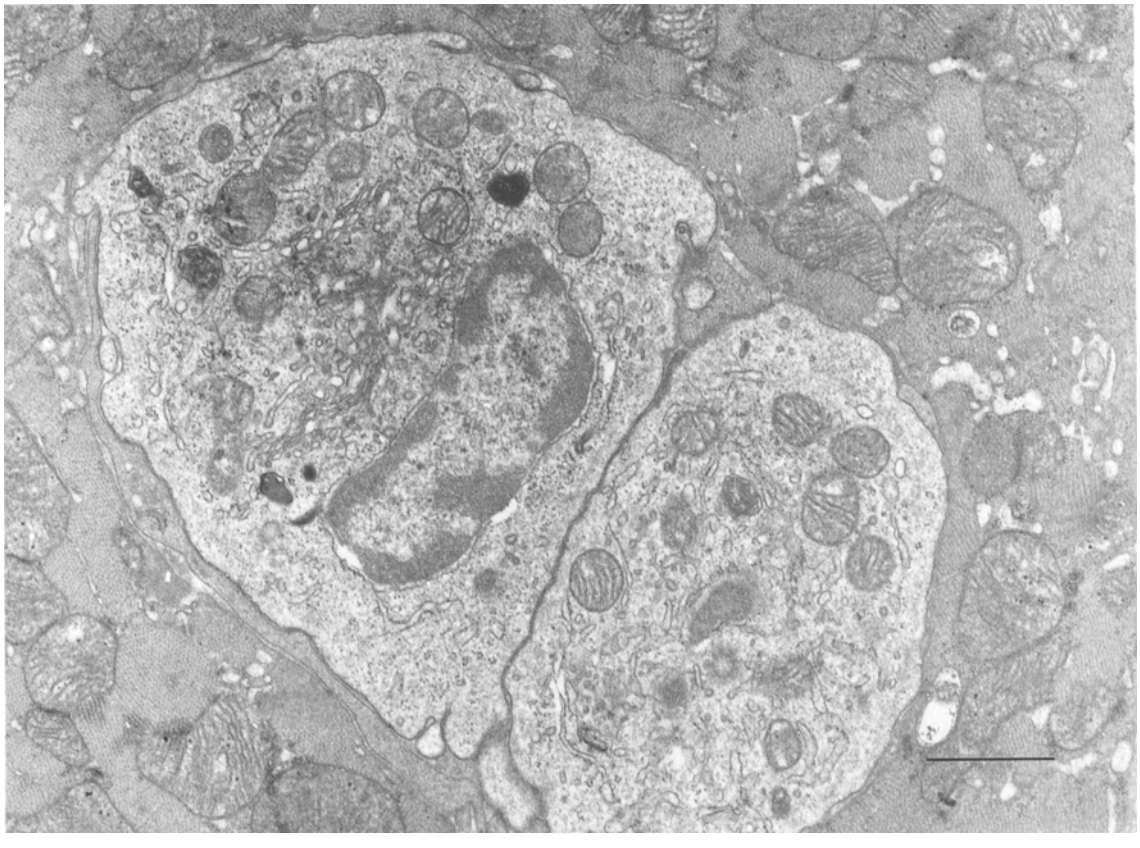

Fig. 7. Electron micrograph of the myocardium in an allograft. Two lymphocytes have penetrated near the centre of the cardiocyte. *Scale bar*, 1 μm

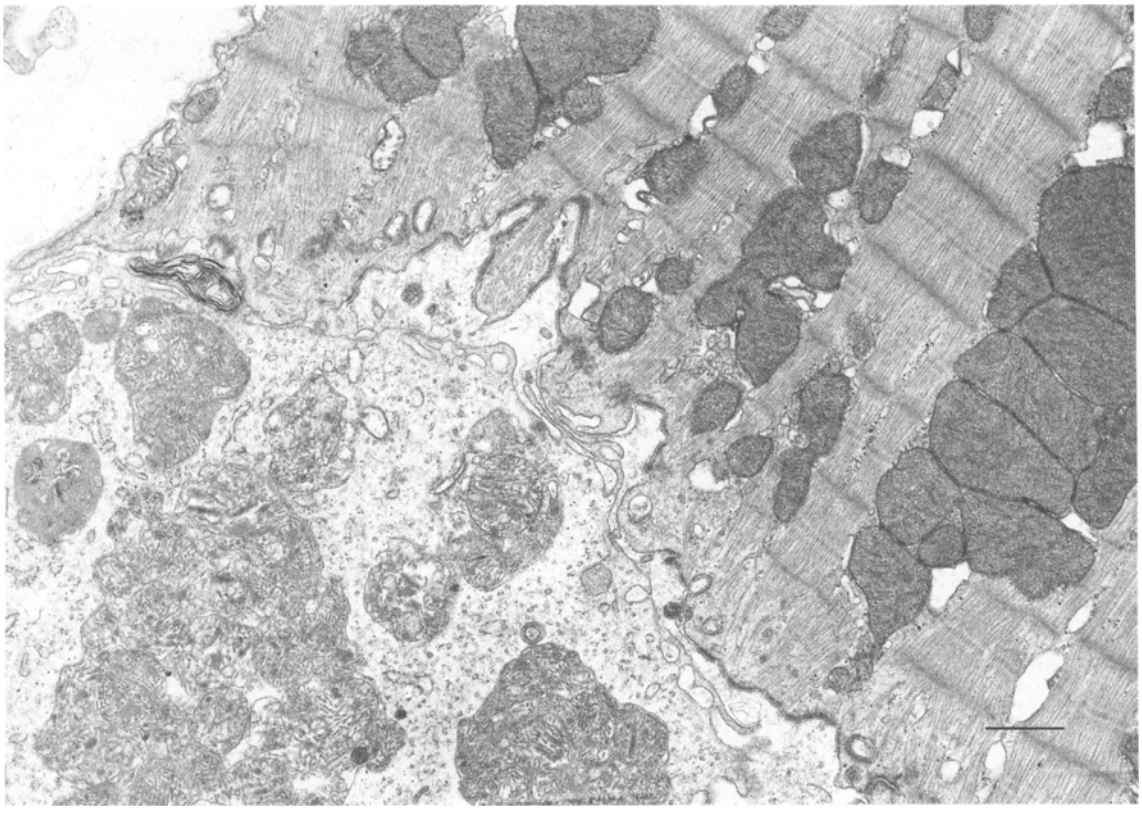

Fig. 8. Electron micrograph of the myocardium in an allograft. One of two cardiocytes joined by an intercalated disc is necrotic and phagocytosed by monocyte/macrophage, whereas the other cell looks viable. *Scale bar*, 1 μm

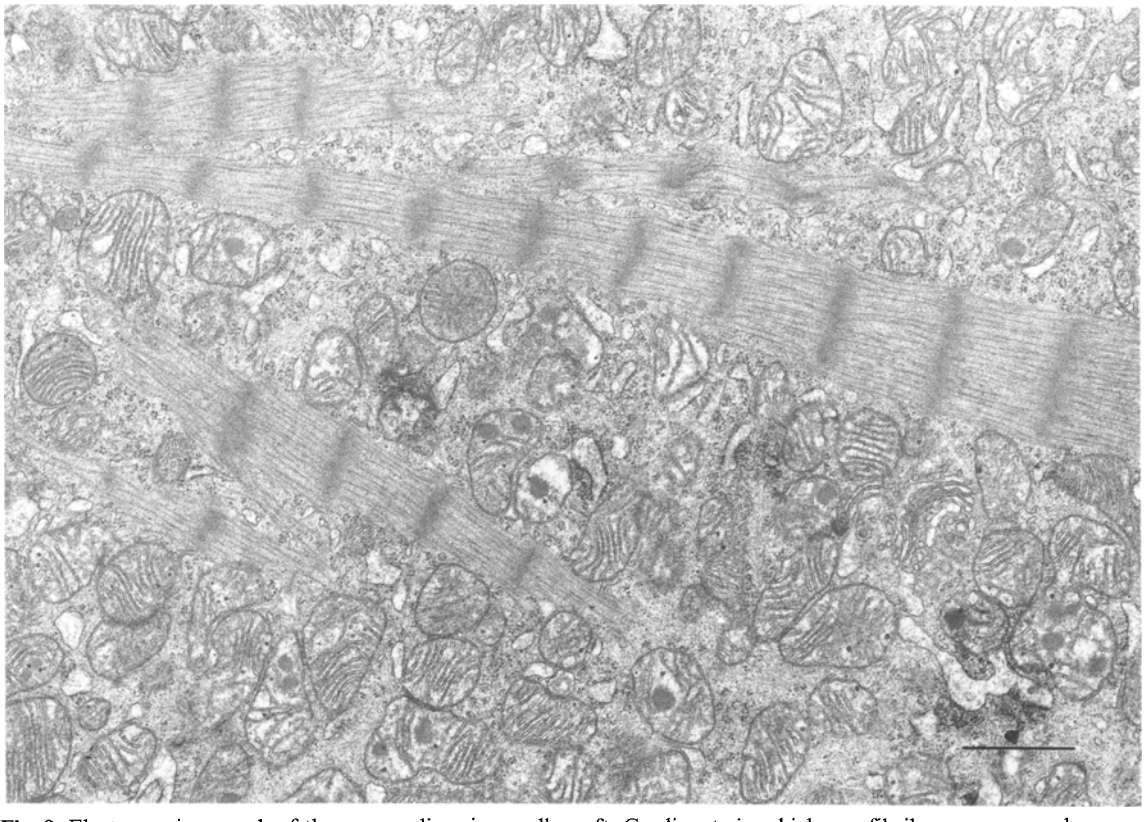

Fig. 9. Electron micrograph of the myocardium in an allograft. Cardiocyte in which myofibrils are scarce and some mitochondria contain moderately electron-dense inclusions. *Scale bar*, 1 μm

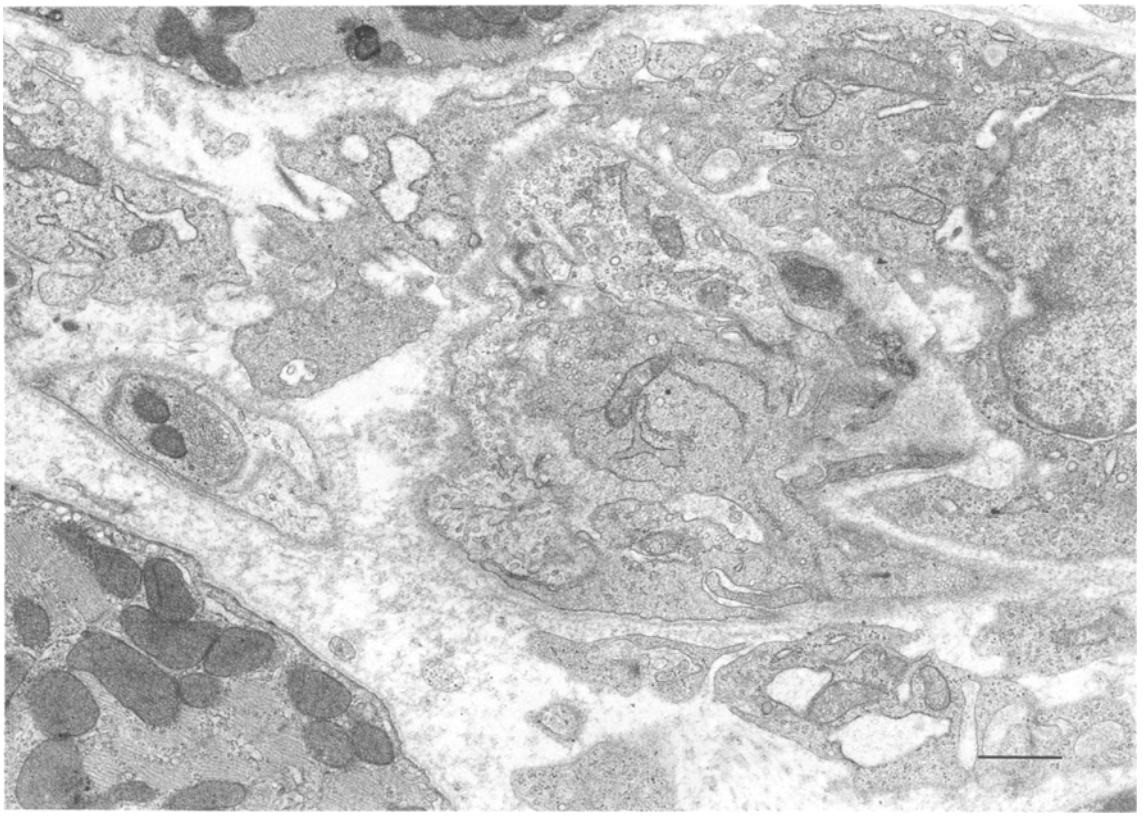

Fig. 10. Electron micrograph of a capillary in an allograft. The lumen is narrowed, and endothelial cellular swelling and an increase of pinocytotic vesicles can be observed. *Scale bar*, 1 μm

were also seen in the widened intracytoplasmic confines of some cardiocytes. It seems that they had invaded from the lateral side of the cardiocyte and approached near to the centre. These two peculiar types of conjugate emperipolesis formation may be involved in cell-mediated cardiocyte injury. Burns et al. (1982) reported that NK cells emperipolesed in human malignant melanoma cells, representing one type of effector-target cell contact leading to cytotoxicity. We have also previously reported that lymphocytes and Mø emperipolesed into cardiocytes in murine coxsackie B3 virus myocarditis (Deguchi et al. 1989). Billingham (1987) pointed out that acute cardiac rejection and primary acute myocarditis may present with similar morphological findings. Our ultrastructural findings are consistent with her hypothesis.

The damaged myocardial sites appeared to increase in area in parallel with the increase on the cellular infiltrate. $IL2R^+$ cells and Tc-s were often in close contact with viable cardiocytes, whereas Mø were often in contact with cardiocytes which had undergone a variety of degenerative changes, including necrosis. Most of the Mø observed in association with degenerated cardiocytes in the late stage of the rejection were phagocytic cells. The necrosis often involved one of two adjacent cells which were joined to each other by an intercalated disc. Occasionally, one of two cardiocytes was necrotic and phagocytosed by Mø, whereas the other looked viable. Tc-s are MHC class 1 restricted cells and bind to target cells bearing the appropriate MHC antigen. After binding, membrane permeability of the target cell changes, and then the cell is disrupted. This MHC recognition by Tc-s may cause the morphological difference in cardiocyte injury.

Even after lysis of the cardiocytes, the major portion of the basement membrane was left behind as a remnant structure. These findings suggest that the basement membrane does not possess much antigenicity in cell-mediated immune cardiocyte injury, and that intimate contact between the plasma membranes of the viable effector cell and the target cell is a prerequisite for effective cytolysis. These changes are also similar to those in viral myocarditis (Deguchi et al. 1989).

There has been much investigation into the subpopulation of circulating T lymphocytes (Liebert et al. 1983; Shapiro et al. 1984). Kawaguchi et al. (1986) reported that a reversed *Th* to Tc-s ratio (less than 1) was associated with a reduced incidence of rejection onset in patients treated with cyclosporine. The ratio often fails to correlate with the histological findings in biopsy specimens. In our sequential analysis, the ratio of *Th* to Tc-s tended to decrease after the transplantation. It was under 1.0 on the 6th and 7th day, when many cardiocytes had degenerated in the allografted hearts. In the analysis of T cell subsets *in situ*, however, the *Th*/Tc-s ratio decreased significantly with time ($r_s = -0.836$, $P < 0.01$) and was under 1.0 from the 4th day. Therefore, *in situ* analysis reflects the morphological changes in the rejection more precisely than the analysis of peripheral blood.

In summary, it is clear that NK cells play a pivotal role in the early stage of the rejection, and that cytotoxic/suppressor T cells and macrophages then aggravate cell-mediated injury. Two types of cardiocyte injury in emperipolesis were identified; one was seen in the widened intercellular spaces of dissociated intercalated discs, and the other in the intracytoplasmic confines of cardiocytes.

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References

Ahmed-Ansari A, Knopf WD, Murphy DA, Tadros T, Leatherbury A, Goodroe JH, Dempsey CL, Gravanis MB, Sell KW (1988) Characterization of human cardiac infiltrating cells post transplantation. 1. Phenotypic and functional alloreactivity. Am J Cardiovasc Pathol 2:193–210

Bellanti JA, Rocklin RE (1985) Cell-mediated immune reactions. In: Bellanti JA (ed) Immunology III. Saunders, Philadelphia, pp 176–188

Billingham ME (1987) Is acute cardiac rejection a model of myocarditis in humans? Eur Heart J 8 [Suppl J]:19–23

Burns ER, Zucker-Franklin D, Valentine F (1982) Cytotoxicity of natural killer cells. Correlation with emperipolesis and surface enzymes. Lab Invest 47:99–107

Christmas SE, MacPherson GG (1982) The role of mononuclear phagocytes in cardiac allograft rejection in the rat. I. Ultrastructural and cytochemical features. Cell Immunol 69:248–270

Cramer DV (1987) Cardiac transplantation: immune mechanisms and alloantigens involved in graft rejection. CRC Crit Rev Immunol 7:1–30

Deguchi H, Kitaura Y, Hayashi T, Kotaka M, Kawamura K (1989) Cell-mediated cardiocyte injury in viral myocarditis of mice and patients. Jpn Circ J 53:61–77

Ellis TM, Lee HM, Mohanakumar T (1981) Alterations in human regulatory T lymphocyte subpopulations after renal allografting. J Immunol 127:2199–2203

Forbes RDC, Lowry RP, Darden AG, Gomersall M, Marghesco DM (1988) Morphologic studies of acute rat cardiac allograft rejection across an isolated major histocompatibility complex class I (RT1A) disparity. Transplantation 45:943–948

Hayashi T, Deguchi H, Kitaura Y, Kawamura K, Otsu I, Nozawa M (1987) Ultrastructural and immunohistochemical study of the myocardium in acute cardiac allograft rejection. Jpn Circ J 51:882

Herscowitz HB (1985) Immunophysiology: cell function and cellular interactions in antibody formation. In: Bellanti JA (ed) Immunology III. Saunders, Philadelphia, pp 117–159

Kawaguchi A, Mohanakumar T, Lee HM, Thimson JA, Lower RR (1986) T-lymphocyte analysis in cardiac allograft recipients treated with cyclosporine. Ann Thorac Surg 42:517–522

Liebert M, Rosenthal JT, Merrall E, Taylor RJ, Singh G, Starzl TE, Rabin BS, Hakala TR (1983) Peripheral blood T lymphocytes found in renal allograft recipients treated with cyclosporine. Transplantation 36:200–202

Lowry RP, Forbes RDC, Blackburn JH, Marghesco DM (1985) Immune mechanisms in organ allograft rejection. Transplantation 40:545–550

Marboe CC, Knowles DM II, Chess L, Reemtsma K, Fenoglio JJ Jr (1983) The immunologic and ultrastructural characterization of the cellular infiltrate in acute cardiac allograft rejection: prevalence of cells with the natural killer phenotype. Clin Immunol Immunopathol 27:141–151

Maugh TH II (1980) New technique for selective immune suppression increase transplant odds. Science 210:44–46

McLean IW, Nakane PK (1974) Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron microscopy. J Histochem Cytochem 22:1077–1083

Ono K, Lindsey ES (1969) Improved technique of heart transplantation in rats. J Thorac Cardiovasc Surg 57:225–229

Rose ML, Yacoub MH (1987) The immunology of cardiac rejection in man. In: Spry CJF (ed) Immunology and molecular biology of cardiovascular diseases. MTP Press, Lancaster, pp 177–197

Rose ML, Gracie JA, Fraser A, Chisholm PM, Yacoub MH (1984) Use of monoclonal antibodies to quantitate T lymphocyte subpopulations in human cardiac allografts. Transplantation 38:230–234

Shapiro R, Goldman MH, Allen C, Brown J, Capehart J, Kawaguchi A, Lower RR (1984) Monitoring of T cell subpopulations in heterotopic cardiac allografts in the rat. Transplant Proc 16:1540–1541

Smith JA, Mottram PL, Miriskalavos A, Mason A, Dumble LJ, Clunie GJA (1987) The effect of operative ischemia in murine cardiac transplantation: isograft control studies. Surgery 101:86–90