

# Interstitial Dendritic Cells of the Rat Heart

## Quantitative and Ultrastructural Changes in Experimental Myocardial Infarction

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**Background.** This study was undertaken to investigate the qualitative and quantitative changes that interstitial dendritic cells (IDC) of the heart undergo during the time course of experimental myocardial infarction.

**Methods and Results.** Left coronary arterial ligations were performed in 43 rats that were killed 2, 4, 7, 14, and 21 days after surgery. Thirteen unoperated and 39 sham-operated rats were used as controls. Frozen sections were stained with monoclonal antibodies (OX 6 and W3/25) to identify and count IDC by light microscopy. Immunoelectron microscopy was also used to identify IDC. The number of IDC per mm<sup>2</sup> of tissue section was calculated for all hearts. In hearts with myocardial infarction, IDC were counted in three areas: the center of the myocardial infarction, the border zone, and the noninfarcted left ventricle. In OX 6 antibody-stained preparations, the number of IDC per mm<sup>2</sup> was  $82 \pm 10$  in the left ventricle of unoperated rats. Hearts with myocardial infarction showed marked increases in the numbers of IDC per mm<sup>2</sup> in the border zone ( $796 \pm 79$  at 7 days and  $528 \pm 98$  at 14 days). In the border zone, IDC often were associated with small clusters of T-helper lymphocytes, which reacted with W3/25 antibody (the rat homologue of human CD4). The center of the myocardial infarction showed an increase in IDC only on day 7 ( $120 \pm 18$ ). By 21 days, IDC in the border zone were only slightly increased ( $159 \pm 15$ ).

**Conclusions.** These findings suggest that IDC migrate to the myocardial infarction border zone. They participate in the activation of lymphocytes and in the initiation of immune responses and decrease in number as inflammation subsides and scarring develops. (*Circulation* 1993;87:909-920)

**KEY WORDS** • immunohistochemistry • MHC antigens • monoclonal antibodies • T-helper lymphocytes

The dendritic cell family comprises several distinct types of cells, including the interstitial dendritic cells (IDC); the interdigitating follicular cells of afferent lymphoid tissues such as the spleen, lymph nodes, and Peyer's patches; and the Langerhans cells of the epidermis.<sup>1,2</sup> Dendritic cells have an important function in the initiation of immune responses because of their role in antigen presentation and in the activation of immune effector cells.<sup>3</sup> IDC are widely distributed in the interstitial connective tissue of most non-lymphoid organs. In the heart, dendritic cells were first described by Hart and Fabre.<sup>3</sup> They are characterized by their slender, elongated cytoplasmic processes that extend in several directions from a thin, centrally located cell body in which a single nucleus is present. Dendritic cells express class II major histocompatibility complex (MHC) antigens (Ia antigens) on their sur-

faces, and this expression provides a basis for the immunohistochemical identification of these cells.<sup>4</sup> Dendritic cells originate in bone marrow<sup>5</sup> and are nonphagocytic in nature. They can be isolated from circulating blood and are relatively short lived in the spleen, lymph nodes, and peripheral lymph. They are replaced by blood-borne precursors<sup>6</sup> and are incapable of further cell division.<sup>7</sup> In rat heart, their turnover time has been estimated to be about 2-4 weeks<sup>3,7</sup> or approximately 25 days.<sup>8</sup> Because of the properties just cited, studies of IDC in diseased hearts would appear to be of special interest. However, investigations of this type have been largely limited to transplanted hearts, in which these cells have been studied in relation to the rejection phenomenon.<sup>9-11</sup> Other implications concerning the role of IDC in immunopathology have been raised by the findings that human immunodeficiency viral antigen is harbored by IDC in the hearts of patients with acquired immunodeficiency syndrome<sup>12</sup> and that *Trypanosoma cruzi* antigens persist in splenic dendritic cells of mice after treatment of experimentally induced interaction with this parasite.<sup>13</sup> Despite the potential importance of these observations, no reports of the ultrastructure of IDC in myocardium have been published, and little information is available concerning the role of these cells in other fundamental pathological processes. This communication reports studies on the

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immunohistochemistry, ultrastructure, and distribution of IDC in normal rat myocardium and the sequential changes that these cells undergo in experimental myocardial infarction.

### Methods

Male Sprague-Dawley rats (Taconic Farms, Inc., Germantown, N.Y.) weighing 175–200 g were used in this study. The animals underwent ligation of the left coronary artery 1 mm distal to its origin ( $n=43$ ) or sham operation ( $n=39$ ), as previously described.<sup>14,15</sup> For the sham operations, a thoracotomy was made, and the heart was exteriorized. Then, a suture was placed around the left coronary artery, as in animals undergoing coronary ligation, but this suture was not tied. The heart was placed back in the thorax, and the surgical procedure was concluded in the usual manner. Thirteen unoperated rats were used as controls. Rats from each group were killed by pentobarbital injection at 2, 4, 7, 14, and 21 days after surgery.

### Light Microscopic Immunohistochemistry

For light microscopic immunohistochemical study (26 rats with coronary artery ligation, 34 with sham operation, and 10 unoperated controls), the hearts were excised and processed as described below for the indirect immunoperoxidase procedure of Steiniger et al.<sup>16</sup> Transverse sections of unfixed hearts were embedded in PolyFreeze Tissue Freezing Medium (Polysciences, Inc., Warrington, Pa.) and snap frozen in isopentane/dry ice. Cryostat sections, 5  $\mu$ m thick, were cut, air dried for 20 minutes at room temperature, fixed in cold absolute ethanol for 10 minutes at 4°C, and washed three times in phosphate-buffered saline (PBS) (pH 7.4). The sections were incubated with the appropriate dilution of primary antibody (OX 1, OX 3, OX 4, OX 6, OX 8, OX 18, W3/13, W3/25, ED 2, or W6/32; see below) ranging from 1:40 to 1:200 in PBS containing 1% bovine serum albumin and 0.1% sodium azide. All antibody incubations were carried out in a moist chamber at 4°C for 1 hour. After three washes with PBS, they were incubated with peroxidase-conjugated rabbit anti-mouse IgG (DAKO, Santa Barbara, Calif.) diluted 1:20 in PBS with 5% inactivated normal rat serum at 4°C for 1 hour, and the sections were washed with 0.1 M Tris-buffered saline (TBS) (pH 7.6). The color was developed with 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 0.01% H<sub>2</sub>O<sub>2</sub> for 10 minutes at room temperature. The sections were counterstained with 1% methyl green for 2 minutes, dehydrated, and mounted with Permount. For positive controls, frozen sections of normal rat spleen were stained concurrently with the sections of the hearts. The interdigitating follicular cells of the spleen provided an easily identifiable, intensely reactive type of dendritic cell. For negative control preparations, sections of all tissues were processed through all the steps of the immunohistochemical staining except for the incubation with the first (primary) antibody, which was omitted. Furthermore, incubation with monoclonal antibody W6/32, which was considered irrelevant, was performed as an additional negative control. In addition, for parallel histological observation, transverse sections of all hearts were fixed in neutral buffered formalin and stained with either hematoxylin and eosin or Masson trichrome methods.

### Electron Microscopic Immunohistochemistry

For electron microscopic immunohistochemical study, the heart tissues from 17 rats with experimental myocardial infarctions, five sham-operated rats, and three unoperated rats were processed according to the indirect peroxidase procedure described by Forbes et al.<sup>17</sup> All rats were perfused with freshly prepared 4% paraformaldehyde and 0.01% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). Pieces of the perfused tissues were immersed in the above fixative for 4 hours at 4°C and then cut into 50- $\mu$ m slices using a Sorvall TC-2 Tissue Sectioner (DuPont Company, Newtown, Conn.). After three successive 10-minute washes with PBS, the tissues were incubated with the primary antibodies (OX 6 or W3/25) overnight at 4°C with agitation. After three washes with PBS, the tissues were incubated with a 1:20 dilution of affinity-purified rabbit antimouse IgG (DAKO) for 2 hours at 4°C with agitation. After an additional three washes, the tissues were reacted with 0.05% DAB in 0.1 M TBS (pH 7.6) for 10 minutes and then treated with 0.05% DAB and 0.01% H<sub>2</sub>O<sub>2</sub> for 15 minutes at room temperature. After washing in distilled water for 10 minutes, the tissues were postfixed in 1% osmium tetroxide for 1 hour, dehydrated in graded alcohols, and embedded in Polybed 812. One-micrometer-thick, toluidine blue-stained sections were studied by light microscopy to select representative areas of tissue for fine structural analysis. Ultrathin sections were examined, with and without additional staining with uranyl acetate, at 60 kV in a JEOL JEM-1200 EX electron microscope.

### Monoclonal Antibodies

Mouse monoclonal antibodies used in this study included OX 1, OX 3, OX 4, OX 6, and W3/25 antibodies, which are known to react with IDC of rat tissues. OX 3 reacts with a polymorphic determinant of rat Ia antigens of AO, Wistar, and Lewis strains<sup>4</sup>; OX 4 and OX 6 react with a common determinant of rat Ia antigens of all strains.<sup>4</sup> However, these antibodies also react with other cells of rat tissues. OX 1 reacts with all rat leukocyte common antigens.<sup>18</sup> OX 6 reacts with B cells and macrophages. W3/25 also reacts with rat T-helper lymphocytes and macrophages.<sup>19,20</sup> This antibody is regarded as being directed against the rat homologue of CD4.<sup>20</sup>

Other antibodies used in this study included OX 18, which reacts with class I MHC antigens of all rat strains<sup>21</sup>; OX 8, which reacts with T-cytotoxic/suppressor subsets of rat T-lymphocytes and natural killer cells<sup>22</sup>; W3/13, which reacts with T-cytotoxic lymphocytes, neutrophils, plasma cells, and some natural killer cells<sup>23</sup>; ED 2, which recognizes tissue macrophages of rat<sup>24</sup>; and W6/32, a mouse monoclonal anti-human HLA-A,B,C (homologous leukocytic antigen) antibody,<sup>25</sup> which was used as a negative control for the specificity of the immunoperoxidase staining. All antibodies were obtained in the form of ascitic fluid. ED 2 antibody was purchased from Bioproducts for Science, Inc., Indianapolis, Ind., and all other antibodies were purchased from Sera-Lab Ltd. (Accurate Chemical and Scientific Corporation, San Diego, Calif.).

### Method for Counting the IDC

A Zeiss Videoplan 2 (Carl Zeiss, Inc., FRG) was used to count the number of IDC. The only cells included in these counts had dendritic morphology and clearly positive reaction for either OX 6 or W3/25 antibody. Neither large nor small round cells were included, because such cells represent macrophages and lymphocytes, respectively. This assumption was validated by electron microscopic study. The number of IDC was counted using a  $200 \times 210\text{-}\mu\text{m}$  ( $0.042\text{ mm}^2$ ) grid at five separate high-power fields ( $\times 400$ ). The resultant data were expressed as mean number  $\pm$  SD of IDC per  $\text{mm}^2$ . For numerical assessments of the frequency of the IDC reactive with OX 6 or W3/25 antibody, the IDC were counted in three different areas in the hearts having myocardial infarcts: the center of the infarct, the border zone of the infarct, and the noninfarcted region of the left ventricle. In the sham-operated and unoperated rats, the IDC were counted in left ventricle, ventricular septum, and right ventricle. The immediate subpericardial and subendocardial regions of the ventricular walls, which could be involved in nonspecific inflammatory changes, were not included in the numerical assessments.

### Statistical Analysis

All data on cell counts are given as mean  $\pm$  SD. The significance of differences in cell counts was evaluated using Student's double-tailed *t* test, with  $p < 0.05$  the level of significant difference.

### Results

#### Immunohistochemical Staining of IDC in Normal Heart

Four of the monoclonal antibodies used in the present study resulted in the immunohistochemical identification of cells with a dendritic type of morphology in myocardial interstitium. These antibodies included OX 3, OX 4, OX 6, and W3/25. OX 3, OX 4, and OX 6 antibodies produced identical results. Antibody W3/25 also produced the same distribution of staining but gave a less intense reaction. This antibody stained approximately 10% fewer dendritic cells than did OX 6 antibody. OX 1 antibody, which is directed against leukocyte common antigen, stained only a few dendritic cells. Ia antigens (as demonstrated by staining with OX 4 and OX 6 antibodies) were found only in the interstitial dendritic cells, not only in the normal hearts but also in the sham-operated and the infarcted hearts; myocytes and endothelial cells were completely negative. OX 8 and W3/13 antibodies failed to give staining of the IDC. OX 18 antibody demonstrated class I MHC antigens in some IDC. Macrophages reacting with ED 2 antibody were rare in the normal hearts. However, in the infarcted hearts, ED 2-positive macrophages increased remarkably. The irrelevant antibody control (W6/32) showed no staining.

#### Histology of Dendritic Cells in Normal Heart

In the normal hearts, IDC exhibited a distinct dendritic morphology, with a centrally located nucleus and long cytoplasmic processes. These cells appeared to be uniformly distributed throughout the heart, with a slight increase in frequency in perivascular areas. In longitudinal sections of myocardium, the IDC appeared elon-

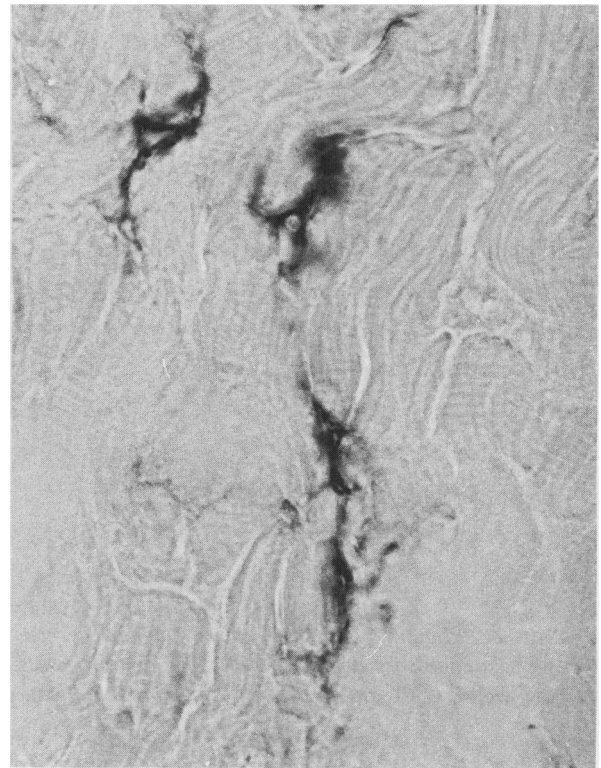


FIGURE 1. Photomicrograph of normal dendritic cells in longitudinal section appearing elongated in shape and showing branching processes. OX 6 antibody stain. Original magnification,  $\times 680$ .

gated in shape, with their long axes generally parallel to those of the adjacent myocytes (Figure 1). In transverse sections, they had irregular or triangular shapes with relatively large nuclei, little cytoplasm, and long processes (Figure 2).

#### Ultrastructure of Dendritic Cells in Normal Heart

In dendritic cells, the electron dense reaction product resulting from staining using the immunoperoxidase method and OX 6 and W3/25 antibodies (Figure 3) revealed labeling limited to the outer surfaces of the plasma membranes but without significant staining of intracytoplasmic or nuclear structures. No preferential staining of any given area of the cell surface was noted. The IDC exhibited flattened elongated nuclei and little cytoplasm. A few cisterns of rough-surfaced endoplasmic reticulum and a few small lysosomes and mitochondria were present. Golgi complexes were inconspicuous, and secretory-type granules were not present. IDC did not contain any Birbeck granules such as those found in Langerhans cells of skin and in the infiltrating cells in Langerhans cell granulomatosis (histiocytosis X).<sup>26</sup> The nuclei had thin, peripheral rims of heterochromatin and small nucleoli. The plasma membranes were smooth with few or no pinocytotic vesicles. IDC did not have demonstrable basement membranes. The cytoplasmic processes of IDC contained few or no organelles and extended between adjacent cells or into the interstitium for variable lengths. No specialized intercellular junctions were observed. Two observations were confirmed at the ultrastructural level: the IDC were located in the

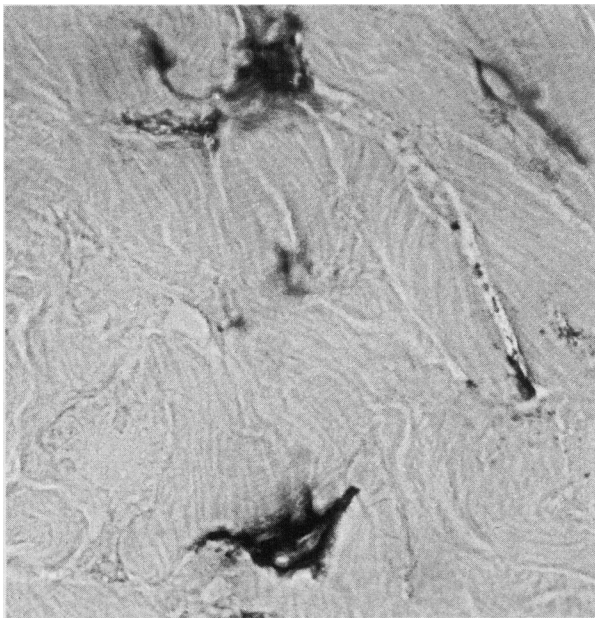


FIGURE 2. Photomicrograph of normal dendritic cells in transverse section showing triangular shape and processes of varying length. OX 6 antibody stain. Original magnification,  $\times 680$ .

interstitial spaces and in the vicinity of capillaries but were not present within areas of splitting of the capillary basement membranes, and cells other than IDC such as fibroblasts and pericytes also had cytoplasmic processes that extended into the myocardial interstitium

and resembled those of IDC. Distinction between these different cell types was best achieved by immunohistochemical staining. The average length and width of the IDC were about  $10\ \mu\text{m}$  and  $2\ \mu\text{m}$ , respectively. There was an average of 3.4 cytoplasmic processes per cell.

#### *Histopathological Observations on Myocardial Infarction*

All hearts examined at 2, 4, 7, 14, and 21 days after coronary ligation demonstrated gross and microscopic changes characteristic of myocardial infarction as described previously.<sup>15</sup> Morphometric studies were not made to determine the exact sizes of these infarcts. However, they were estimated to involve from 20% to 35% of the left ventricle, which is in agreement with the data presented by Fishbein et al<sup>15</sup> and Anversa et al.<sup>27</sup> Within this range of variations, no relation was observed between the sizes of the infarcts and the qualitative and quantitative changes described below in dendritic cells.

Microscopically, the infarcts were characterized by a central, hypereosinophilic zone of coagulation necrosis. The boundary between this zone and the surrounding normal myocardium consisted of a "border zone" in which necrotic and viable myocytes were present together with inflammatory infiltrates. The border zones varied in size and cellular composition according to the age of the infarct. They were present primarily along the lateral edges of these lesions and ranged in size from 100 to  $500\ \mu\text{m}$  at 2 days after coronary ligation. At subsequent times, the border zones containing a high density of inflammatory cells became less sharply delin-

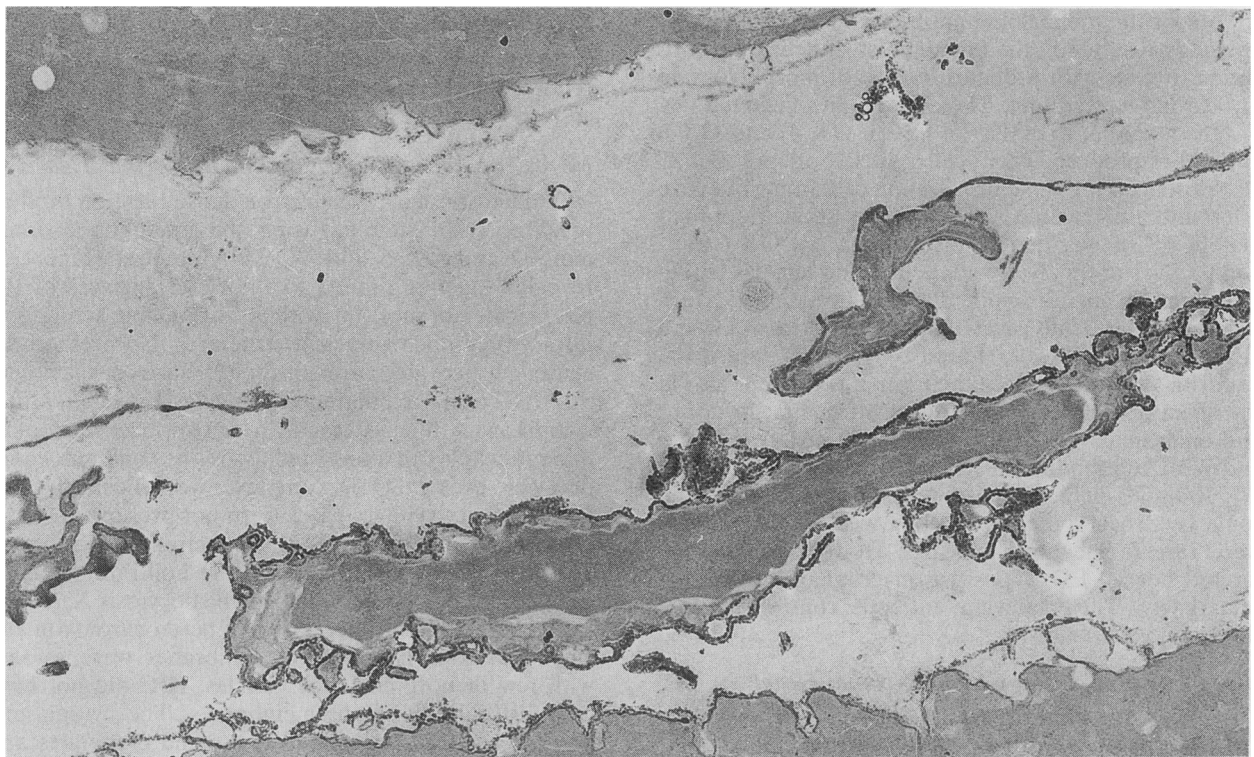


FIGURE 3. Electron micrograph showing characteristic dendritic morphology of a normal dendritic cell. There is significant OX 6 antibody-positive staining on the outer surface of plasma membranes. The widened interstitial space represents an artifact of tissue preparation. Original magnification,  $\times 16,000$ .





FIGURE 4. Photomicrograph of dendritic cells in the center of 7-day-old infarct. They are very thin and have a few processes. OX 6 antibody stain. Original magnification,  $\times 430$ .

eated. The cellular infiltrates consisted mainly of neutrophils and mononuclear cells on day 2; neutrophils, fibroblasts, and proliferating capillaries on day 4; and mononuclear cells and fibroblasts on days 7 through 21. In the sham-operated rats, postoperative adhesions were present on the pericardium. A nonspecific epicarditis, secondary to the surgical procedure of sham operation, was found microscopically.

#### *Morphology of Dendritic Cells in Infarcted Rat Hearts*

**Light microscopy.** The changes that IDC underwent during the time sequence of events after myocardial infarction varied according to whether such cells were present in the center of the myocardial infarction, in the border zone, or in the noninfarcted portion of the left ventricle. Furthermore, for quantitative purposes (see below), it was necessary to make comparisons with the hearts of simultaneously sham-operated rats. On day 2, there was a small decrease in the number of dendritic cells in the infarcted area. The number of dendritic cells in the border zone increased at 4 days and became maximal at 7 days, after which it decreased progressively. On days 2 through 21 after ligation, IDC in the center of the infarct were very thin, and their dendritic appearance was not nearly as marked (Figure 4). In the border zone of 4-day-old infarcts, IDC appeared elongated (Figure 5). In the border zone of 7-day-old infarct, they had more irregular shapes and longer processes, more abundant cytoplasm, and larger nuclei than in normal tissue (Figure 6). On days 14 through 21 after

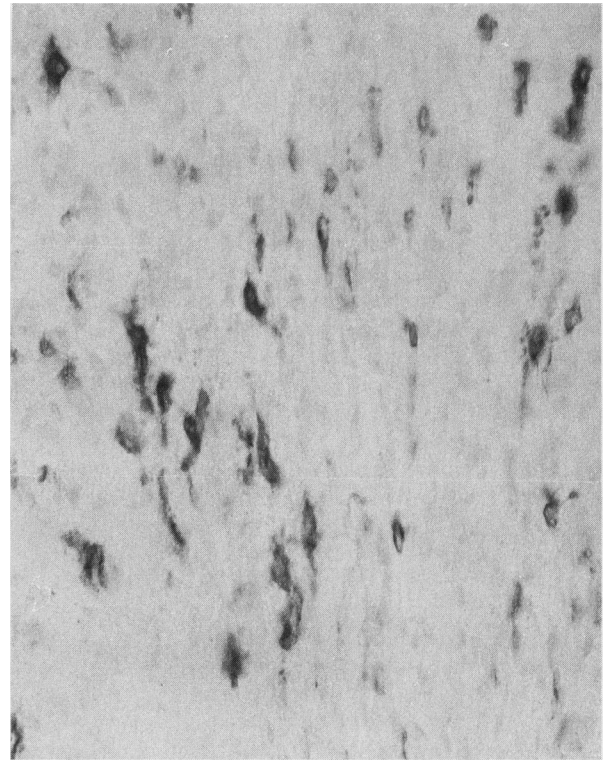


FIGURE 5. Photomicrograph of OX 6 antibody-positive dendritic cells in the border zone of 4-day-old infarct; the numbers are increased. Original magnification,  $\times 430$ .

ligation, IDC in the border zone became more elongated and thin (Figures 7 and 8). Dendritic cells in the border zone frequently were associated with lymphocytes. These lymphocytes were small or medium sized and round and had thin rims of cytoplasm. Most of these lymphocytes were identified as T-helper cells (W3/25 antibody positive); only a few cytotoxic/suppressor T-lymphocytes (OX 8 antibody positive) were found in these areas. On day 2, few T-helper cells were present in the border zone, and only an occasional IDC-lymphocyte aggregate was identified. On day 4, T-helper cells were slightly increased. The smallest IDC-lymphocyte aggregates consisted of three to five lymphocytes and one or two IDC. Aggregates of this type were most prominent in the border zone of 7-day-old infarcts, in which they became larger and more compact, consisting of 10–20 lymphocytes and several IDC (Figure 9). On day 14, groups of only two to three T-helper cells were associated with IDC. On day 21, the IDC were isolated and appeared scattered in the dense connective tissue of the scar. Few T-helper cells were found. In noninfarcted portions of the left ventricle, the morphological characteristics of OX 6 antibody-positive IDC were comparable to those observed in normal and sham-operated hearts.

As shown by staining with OX 18 antibody, class I MHC antigens were expressed on the surfaces of both IDC and endothelial cells of capillaries and large vessels. The membranes of myocytes did not stain for class I MHC antigens. By the 7th day after ligation, OX 18-positive cells occurred more frequently in the border zone than in the centers of the infarcts and the nonin-

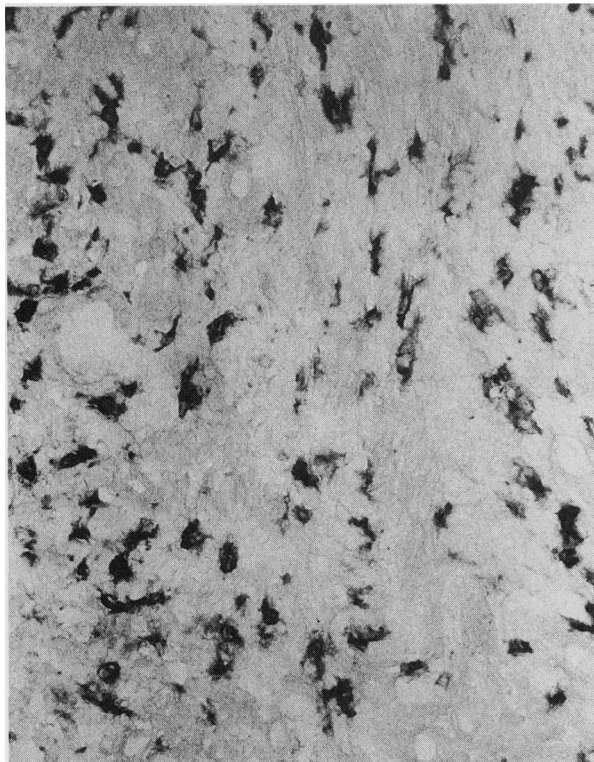


FIGURE 6. Photomicrograph of OX 6 antibody-positive dendritic cells in the border zone of 7-day-old infarct; they are enlarged and greatly increased in number. Original magnification,  $\times 430$ .

farcted portions of left ventricle. No significant changes were found in the distribution of class I MHC antigens in sham-operated hearts and in noninfarcted areas of left ventricles.

**Electron microscopy.** On day 7 after ligation, the IDC in the areas of infarction became clearly distinguishable from those in normal hearts because they showed marked alterations in their size and their dendritic appearance. In the border zones, the IDC became large and plump and had a profusion of long, slender, and branching cytoplasmic processes (Figure 10). Their nuclei became more irregular and prominent, with more abundant heterochromatin and enlarged nuclei. The cytoplasm occupied a narrow zone around the nucleus and was filled with scattered mitochondria, lysosomes, a few multivesicular bodies, vacuoles, and short cisterns of rough-surfaced endoplasmic reticulum. Free ribosomes were relatively few in number and usually occurred as scattered polysomes. The average length and width of the IDC in the border zone were about 13 and 4  $\mu\text{m}$ , respectively. There was an average of 18 cytoplasmic processes per cell. Another remarkable change observed in the border zone was the presence of IDC within the lumina of capillaries, especially in 7-day-old infarcts. These IDC had an irregular shape, numerous cytoplasmic processes, and few organelles. These cells were identified as IDC by staining with OX 6 antibody (Figure 11). In the central regions of the infarcts, the IDC became more elongated and more narrow in width. In the noninfarcted left ventricle of rats undergoing

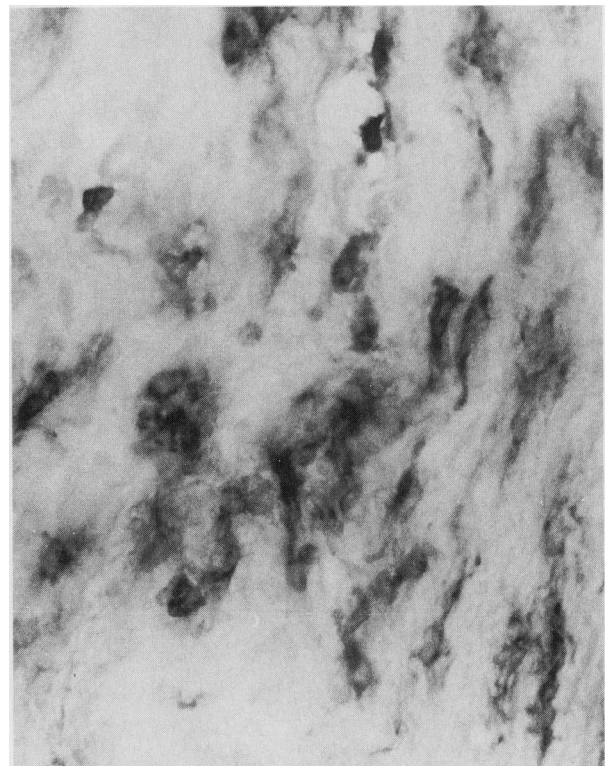


FIGURE 7. Photomicrograph of OX 6 antibody-positive dendritic cells in the border zone of 14-day-old infarct; the numbers are increased. They appear more elongated. Original magnification,  $\times 680$ .

coronary artery ligation, the morphology of IDC was comparable to that of normal IDC (Figure 12).

On day 14, the morphology of the IDC in the border zone showed some unusual features. A few IDC retained their dendritic shapes, with three to eight branching cytoplasmic processes; however, others became bipolar and lacked branching processes. Organelles were reduced in number. At 21 days after ligation, the IDC in the border zone were extremely thin, long, and bipolar. The dendritic appearance had disappeared completely. They were arranged in the spaces between the parallel collagenous bundles. A flattened nucleus occupied nearly the whole body of the IDC. The nucleus had finely dispersed chromatin and indistinct nucleoli. There was a little thin rim of cytoplasm in which organelles could be rarely discerned.

#### *Dendritic Cell Counts in Normal and Infarcted Hearts*

Two different antibodies—OX 6 and W3/25—were used for the cell counts. The numerical results obtained with OX 6 antibody were consistently about 10% higher than those obtained with W3/25 antibody. This was true not only in normal hearts but also in infarcted hearts and in sham-operated hearts (Tables 1 and 2). In the left ventricle of normal rats, a total of  $82 \pm 10$  cells/ $\text{mm}^2$  of tissue section were detected after staining with OX 6 antibody; similar numbers of cells were found in ventricular septum and right ventricle. Tables 1 and 2 show the changes in cell counts after coronary ligation. These changes are presented according to the location of the

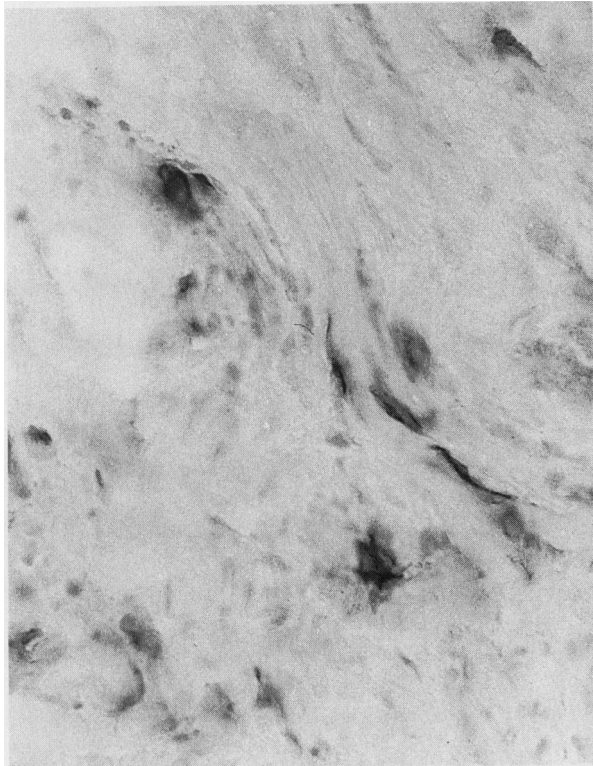


FIGURE 8. Photomicrograph of a few, thin, elongated dendritic cells in the border zone of 21-day-old infarct. OX 6 antibody stain. Original magnification,  $\times 430$ .

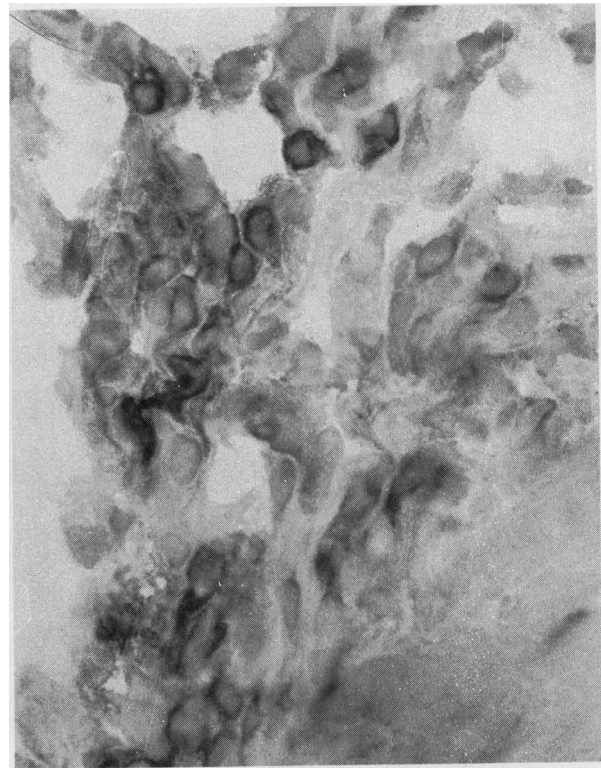


FIGURE 9. Photomicrograph of large cluster formed by dendritic cells and T-helper lymphocyte in the border zone of 7-day-old infarct. W3/25 antibody stain. Original magnification,  $\times 680$ .

area in the center of the infarct, the border zone, or the noninfarcted portion of the left ventricle. In the center of the infarct, the numbers of IDC per  $\text{mm}^2$  decreased at 2 ( $52 \pm 7$ ) and 4 ( $44 \pm 15$ ) days after coronary ligation but increased at 7 days ( $120 \pm 18$ ), returned to normal at 14 days, and became much lower than normal ( $26 \pm 8$ ) at 21 days. In the border zone, the numbers of IDC per  $\text{mm}^2$  showed no significant changes at 2 days. However, at 4 days they showed a marked increase ( $268 \pm 97$ ), which became maximal at 7 days ( $796 \pm 79$ ). These numbers began to decrease at 14 days ( $528 \pm 98$ ) and continued to decrease at 21 days ( $159 \pm 15$ ). In contrast to these changes, the cell counts in the noninfarcted portions of the left ventricle showed mild decreases at 2 and 4 days ( $60 \pm 7$  and  $54 \pm 17$ ). After a mild increase at 7 days ( $128 \pm 14$ ), the cell counts became normal at 14 days and subnormal at 21 days ( $38 \pm 13$ ). In the left ventricles of the sham-operated animals, the cell counts showed an increase at 4 days, a peak increase at 7 days ( $171 \pm 35$ ), and subnormal values at 21 days ( $42 \pm 13$ ).

### Discussion

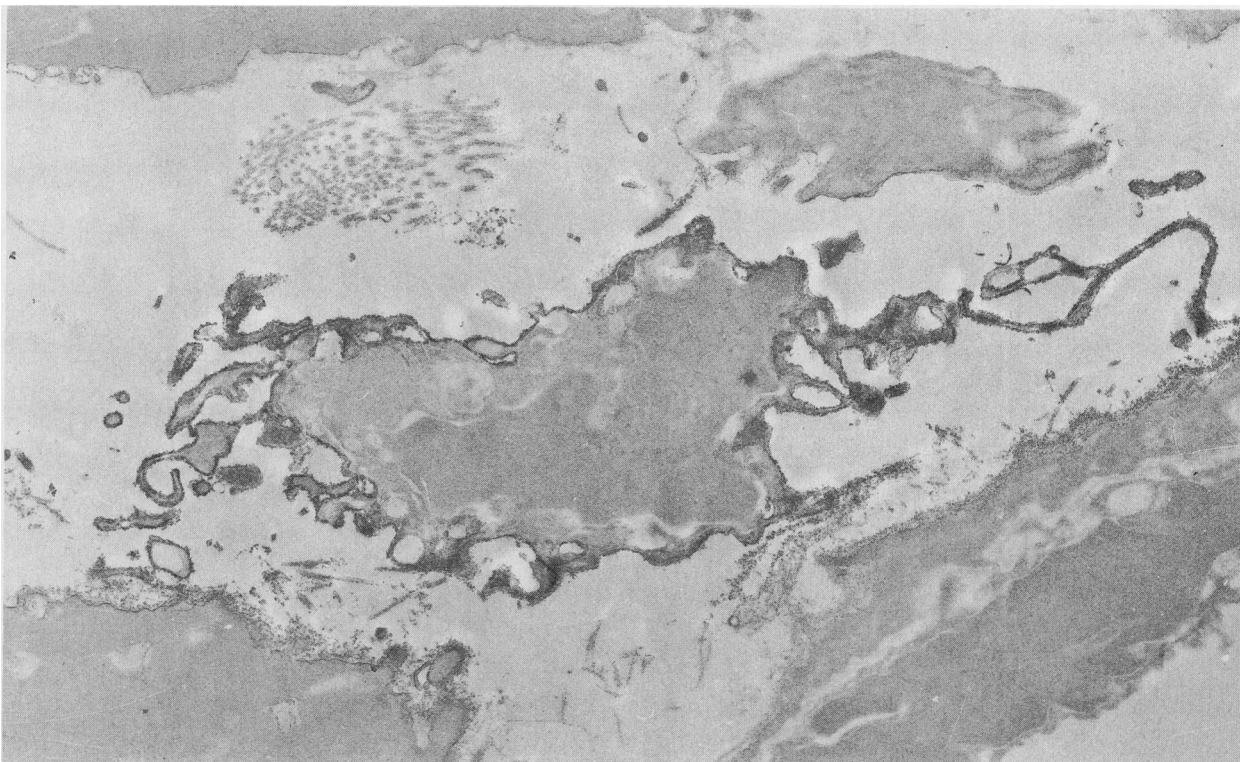
#### *Morphology of Myocardial Dendritic Cells*

The IDC of the heart constitute a distinct type of cell, which differs from macrophages, lymphocytes, smooth muscle cells, and other types of vascular and perivascular cells of the heart. In the present study, distinction of dendritic cells from macrophages was clearly made on the basis of the histological, immunohistochemical, and ultrastructural characteristics. IDC showed no evidence of phagocytic activity at any time period after myocardial infarction. Such activity was very prominent in

macrophages. These cells were easily identified by their round shapes, large sizes, and content of lysosomes and residual bodies. Dendritic cells did not react with ED 2 antibody, which serves as a specific marker for macrophages.<sup>24</sup>

Distinction from lymphocytes was also evident on a morphological basis, i.e., the small sizes, rounded shapes, and typical nuclei of lymphocytes. Our study also showed that IDC tended to become associated with T-helper lymphocytes as part of their response to myocardial infarction. The extensive surface area of IDC allowed single cells of this type to interact directly with a relatively large number of potential responder cells such as T-lymphocytes. Differentiation between IDC and smooth muscle cells was based on the facts that the latter cells are invested by a basement membrane and their cytoplasm contains peripherally located dense bodies that serve as insertion sites for the actin filaments. Smooth muscle cells lacked the surface markers of IDC (OX 6 and W3/25 antibodies). The cytoplasmic processes of pericytes have a resemblance to those of IDC. However, the cytoplasmic processes of pericytes could be traced back to cell bodies located within splits in the basement membranes of capillaries. In this classic location for pericytes, we have not found cells that could be labeled immunohistochemically with antibodies for dendritic cells. Fibroblasts were recognized by their numerous cisterns of rough-surfaced endoplasmic reticulum and prominent Golgi complexes. The cytoplasmic processes of pericytes and fibroblasts in myocardial interstitium remained unreactive in preparations stained with OX 6 antibody.

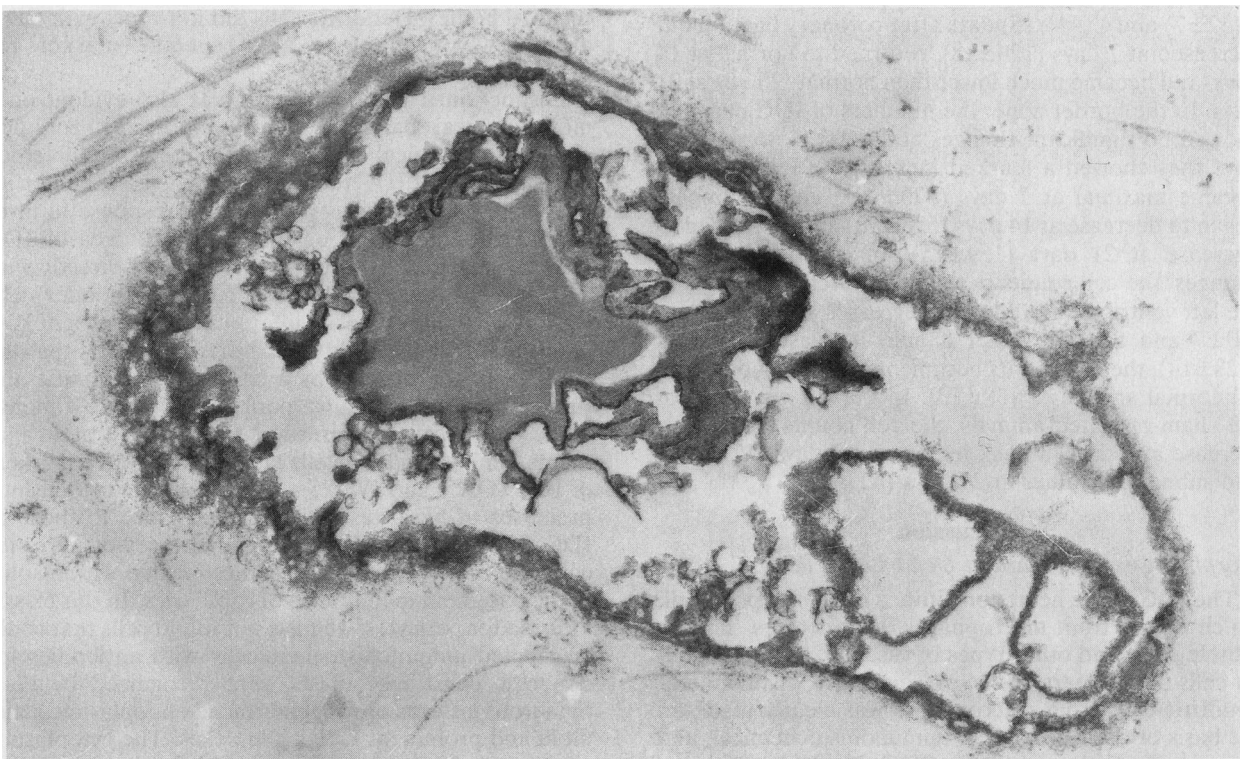




**FIGURE 10.** Electron micrograph of large, plump dendritic cell with a profusion of long, slender, branching cytoplasmic processes and large nucleus in the border zone of 7-day-old infarct. OX 6 antibody stain. Original magnification,  $\times 20,000$ .

A functional heterogeneity of IDC was suggested by our finding in the variability in the cell counts obtained with different monoclonal antibodies. The counts with

OX 6 antibody were consistently higher than those obtained with W3/25 antibody. Furthermore, a significant proportion of the IDC failed to react with OX 1



**FIGURE 11.** Photomicrograph of dendritic cell in the lumen of a capillary of 7-day-old infarct with a typical dendritic appearance. OX 6 antibody stain. Original magnification,  $\times 16,500$ .



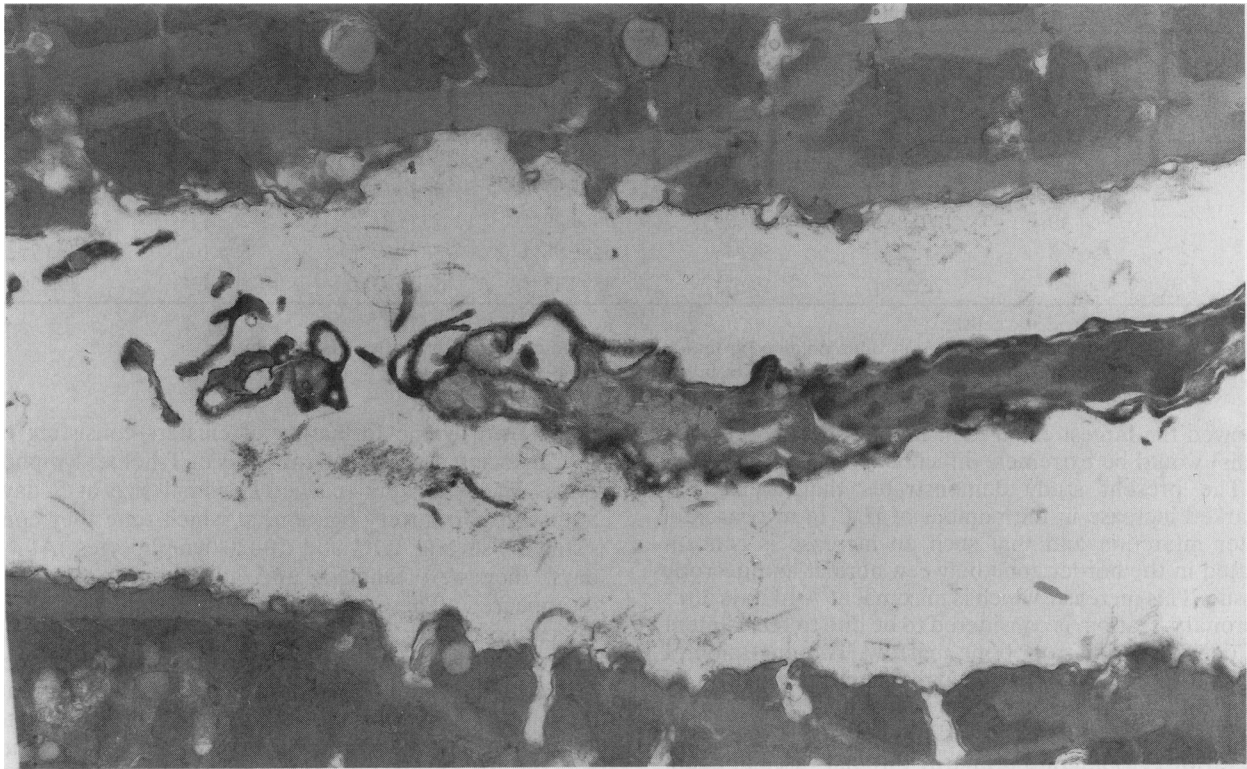


FIGURE 12. Electron micrograph of a dendritic cell in noninfarcted left ventricle of 7-day-old infarct. This cell has a normal appearance. OX 6 antibody stain. Original magnification,  $\times 10,000$ .

antibody (common leukocyte antigen). A similar heterogeneity was noted in a previous study of dendritic cells in rat.<sup>16</sup> This heterogeneity may reflect the fact that IDC express different antigenic markers at different stages during their differentiation from precursor cells.<sup>5,28</sup>

The two most remarkable structural features of the dendritic cells are the paucity of cytoplasmic organelles and the very large area occupied by the plasma membrane relative to the internal volume of the cell. The scarcity of organelles is in accord with the concept that these cells do not engage in a high degree of metabolic activity directed toward protein synthesis, as is the case with fibroblasts and plasma cells. The cell surface is thought to function in antigen presentation, and it seems likely that one of the mechanisms by which dendritic cells modulate this function is by altering their surface area as they become activated. The alteration of the IDC surface in this study was consistent with the

notion that antigens in tissues can be picked up, processed, presented, and transported by dendritic cells via the plasma membrane.<sup>29</sup>

#### Quantitative Alterations in Myocardial IDC After Myocardial Infarction

The results of the cell count in the present study are presented as actual numbers of cells per mm<sup>2</sup> of tissue section. No attempt was made to correct these values for changes in cell geometry for losses of tissue volume resulting from the infarction.<sup>30</sup> The nature and extent of such corrections would vary according to the time elapsed after infarction and the zone of the heart (normal, border, or infarct) in which the cell counts are made. This is particularly true of the border zone, which is more poorly demarcated than the other zones. Calculations of loss of tissue volume in this zone (which

TABLE 1. Frequency of Interstitial Dendritic Cells in Hearts of Sham-Operated and Normal Rats

Days after sham operation	No. of animals	No. ( $\pm$ SD) of OX 6 or W3/25 antibody-positive IDC per mm <sup>2</sup> of tissue section					
		Left ventricle		Ventricular septum		Right ventricle	
		OX 6	W3/25	OX 6	W3/25	OX 6	W3/25
2	2	69 $\pm$ 3	50 $\pm$ 10	67 $\pm$ 7	52 $\pm$ 13	62 $\pm$ 7	48 $\pm$ 9
4	5	101 $\pm$ 27	94 $\pm$ 30*	97 $\pm$ 18*	90 $\pm$ 17*	92 $\pm$ 19	87 $\pm$ 11*
7	10	171 $\pm$ 35*	168 $\pm$ 26*	168 $\pm$ 33*	173 $\pm$ 30*	160 $\pm$ 30*	153 $\pm$ 43*
14	33	105 $\pm$ 16*	73 $\pm$ 3	106 $\pm$ 15*	79 $\pm$ 7	105 $\pm$ 16*	78 $\pm$ 10*
21	14	42 $\pm$ 13*	36 $\pm$ 18*	40 $\pm$ 18*	35 $\pm$ 16*	39 $\pm$ 22*	36 $\pm$ 18*
Normal	10	82 $\pm$ 10	63 $\pm$ 10	79 $\pm$ 11	64 $\pm$ 14	82 $\pm$ 13	60 $\pm$ 8

IDC, interstitial dendritic cells.

\*Significantly different ( $p < 0.05$ ) from value obtained from same area in normal rat.

TABLE 2. Frequency of Interstitial Dendritic Cells in Infarcted Rat Hearts

Days after ligation	No. of animals	No. ( $\pm$ SD) of OX 6 or W3/25 antibody-positive IDC per mm <sup>2</sup> of tissue section					
		Center of infarct		Border zone		Noninfarcted left ventricle	
		OX 6	W3/25	OX 6	W3/25	OX 6	W3/25
2	3	52 $\pm$ 7†	33 $\pm$ 13†	81 $\pm$ 17	67 $\pm$ 19	60 $\pm$ 7†	51 $\pm$ 12
4	6	44 $\pm$ 15*†	44 $\pm$ 13*†	268 $\pm$ 97*†	234 $\pm$ 100*†	54 $\pm$ 17*†	52 $\pm$ 9*†
7	10	120 $\pm$ 18*†	119 $\pm$ 29*†	796 $\pm$ 79*†	735 $\pm$ 83*†	128 $\pm$ 14*†	116 $\pm$ 14*†
14	3	81 $\pm$ 13	50 $\pm$ 15	528 $\pm$ 98*†	387 $\pm$ 19*†	89 $\pm$ 10	78 $\pm$ 7†
21	4	26 $\pm$ 8*†	19 $\pm$ 4†	159 $\pm$ 15*†	119 $\pm$ 13*†	38 $\pm$ 13†	30 $\pm$ 5†

IDC, interstitial dendritic cells.

\*Significantly different ( $p<0.05$ ) from value obtained from left ventricle at period of time after sham operation.†Significantly different ( $p<0.05$ ) from value obtained from left ventricle of normal rat.

showed the largest changes in the number of dendritic cells) would be extremely difficult.

The present study demonstrates that there is a marked increase in the number of IDC in myocardium after infarction and that such an increase is concentrated in the border zone between normal and necrotic tissue. This increase, which is maximal at 7–14 days after coronary ligation, is considered to be due to recruitment of dendritic cells from bone marrow. The migration of dendritic cells was suggested by our observations of such cells within the lumina of myocardial capillaries in the border zones. These findings further suggest that dendritic cells migrate primarily through vascular channels and that they have only a very limited ability to penetrate into areas, such as the central regions of myocardial infarcts, that do not have a functional microvascular bed. We consider this to be the reason why IDC accumulate at the boundary between viable and necrotic myocardium.

The increase in the number of IDC was accompanied by an increase in their overall sizes and in the number of their cytoplasmic processes. This resulted in a marked increase in the surface area available for expression of membrane antigens. Together with this, there also was an increase in the sizes and numbers of the intracellular organelles of dendritic cells. However, these organelles continued to be relatively few and small.

A relatively small but significant decrease in the number of dendritic cells per mm<sup>2</sup> of tissue section was observed in the central regions of 2-day-old infarcts. This decrease may have been due to the combined effects of a normal degree of loss of IDC resulting from cell turnover, plus interruption of tissue influx of these cells via the circulation; myocardial infarction causing the death of some IDC (as it causes death of at least some vascular and connective tissue cells); and coexistence of both of these factors. The data obtained in the present study did not allow this problem to be analyzed.

We believe that the increase in the number of IDC in sham-operated hearts represents a diffused myocardial response to the considerable trauma associated with the sham operation. The subsequent decrease in the number of IDC in sham-operated hearts to values below those found in unoperated hearts is interpreted as an "overshoot" in the downregulation of this initial inflammatory response.

#### Activation of T-Lymphocytes by IDC in Myocardial Infarction

The most clear evidence of activation of T-lymphocytes by IDC in the heart after myocardial infarction

was shown by the formation of clusters consisting of IDC associated with small numbers of T-helper lymphocytes. These clusters reached maximal size at 7 days after coronary artery ligation, at which time they consisted of several IDC and 10–20 lymphocytes. At 14 days, they were smaller, and at 21 days, they had disappeared. These observations are interpreted as indicating that dendritic cells migrate from the bone marrow to the heart, evidently in response to chemotactic stimuli, after myocardial necrosis develops, and they present heart-derived antigenic components to T-lymphocytes. As necrosis and inflammation subside, the recruitment of dendritic cells ceases, and the IDC that remain in the border zone assume a less complex appearance as healing by fibrosis takes place.

Staining with monoclonal antibody W3/25 demonstrated that the increase in the number of IDC was accompanied by numerous T-helper lymphocytes, primarily at sites of focal cellular infiltration in the border zones of the infarcts. This finding is similar to that reported by Forbes et al,<sup>17</sup> who demonstrated that Ia antigen reactive T-helper cells in rat cardiac allografts were in close apposition to dendritic cells. Coexistence of, and contacts between, lymphocytes and dendritic cells have been found in the dermal infiltrate in Sezary syndrome<sup>31</sup> and in rheumatoid arthritis.<sup>32</sup> This clustering phenomenon is believed to be important for cell-to-cell interaction and subsequent T-cell activation and proliferation.<sup>32</sup> It is well known that the clustered T-cells can produce interleukin-2, whereas the nonclustered T-cells do not.<sup>33</sup> Therefore, we assume that the aggregates of IDC and T-helper lymphocytes found in this study represent the counterpart of dendritic cell-lymphocyte clustering in vivo, in which these T-helper cells would release cytokines, particularly interleukin-2. When these dendritic cells are mixed with T-helper cells, the resulting aggregates may play an important role in T-cell growth and differentiation.

Histochemical staining with monoclonal antibody OX 8 demonstrated a mild increase in T-cytotoxic/suppressor lymphocytes in the cellular infiltrates of the border zones of 7-day-old infarcts. The present study indicates that the increase in the number of T-cytotoxic/suppressor cells was accompanied by an increase in the number of OX 18-positive (i.e., positive for class I MHC antigens) dendritic cells. T-cytotoxic/suppressor lymphocytes are specific for class I MHC antigens, by which they are activated. The present study also shows that cytotoxic T-lymphocytes were much less numerous

than T-helper cells in the border zone of 7-day-old infarcts. Similar results were reported in rat cardiac allograft rejection.<sup>17,34</sup> Inaba et al<sup>35</sup> have shown that the responses of cytotoxic and helper T-cells to dendritic cells are comparable in many respects. Both types of T-lymphocytes form clusters with dendritic cells and then release cytokines, particularly interleukin-2. The present study did not identify aggregates of dendritic cells and T-cytotoxic/suppressor lymphocytes because OX 8 antibody did not label IDC, in contrast to W3/25 antibody, which labels both IDC and T-helper lymphocytes.

As mentioned previously, dendritic cells produce cytokines and induce other cells to produce these agents, including not only interleukin-1 but also interleukin-2 and tumor necrosis factor.<sup>36</sup> The production of interleukin-1 by dendritic cells remains the subject of controversy.<sup>37,38</sup> The relations between cytokines and dendritic cells need to be evaluated in the context of recent studies showing that interleukin-1, interleukin-2, and tumor necrosis factor are capable of inducing endothelial cell and myocyte damage, myocardial inflammation, increased vascular permeability, interstitial edema, and depression of myocardial function.<sup>39–41</sup> Taken together, these observations suggest that the accumulations of IDC at the margins of infarcts may have detrimental effects on cardiac function.

In summary, the present study shows that interstitial IDC migrate to the heart, presumably from the bone marrow, after myocardial infarction and become concentrated in the border zone of the infarct, where they become associated with T-helper lymphocytes. IDC are not phagocytic, and they are thought to function in the initiation of the immune response and in the recruitment and activation of other types of inflammatory cells. By 21 days after infarction, the inflammatory process has subsided, and the IDC have assumed slender, bipolar configurations that differ from their usual dendritic appearances and are considered to be suggestive of an inactive state.

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