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Leukocytes express connexin 43 after activation with lipopolysaccharide and appear to form gap junctions with endothelial cells after ischemia–reperfusion

(intercellular communication/inflammation/polymorphonuclear granulocytes)

PATRICIO I. JARA, MAURICIO P. BORIC, AND JUAN C. SÁEZ

Departamento de Ciencias Fisiológicas, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Alameda 340, Santiago, Chile

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ABSTRACT **Levels and subcellular distribution of connexin 43 (Cx43), a gap junction protein, were studied in hamster leukocytes before and after activation with endotoxin (lipopolysaccharide, LPS) both *in vitro* and *in vivo*. Untreated leukocytes did not express Cx43. However, Cx43 was clearly detectable by indirect immunofluorescence in cells treated *in vitro* with LPS (1 μg/ml, 3 hr). Cx43 was also detected in leukocytes obtained from the peritoneal cavity 5–7 days after LPS-induced inflammation. In some leukocytes that formed clusters Cx43 immunoreactivity was present at appositional membranes, suggesting formation of homotypic gap junctions. In cell homogenates of activated peritoneal macrophages, Cx43, detected by Western blot analysis, was mostly unphosphorylated. A second *in vivo* inflammatory condition studied was that induced by ischemia–reperfusion of the hamster cheek pouch. In this system, leukocytes that adhered to venular endothelial cells after 1 hr of ischemia, followed by 1 hr of reperfusion, expressed Cx43. Electron microscope observations revealed small close appositions, putative gap junctions, at leukocyte–endothelial cell and leukocyte–leukocyte contacts. These results indicate that the expression of Cx43 can be induced in leukocytes during an inflammatory response which might allow for heterotypic or homotypic intercellular gap junctional communication. Gap junctions may play a role in leukocyte extravasation.**

Activation of circulating leukocytes occurs under diverse conditions that lead to an inflammatory response. At sites of inflammation, the arrest of circulating leukocytes that migrate from blood into the parenchyma is favored by induction of the expression of cell adhesion molecules on the surface of leukocytes and endothelial cells (1, 2). These cell surface receptors include P- and E-selectin (3–5) and integrins (6, 7).

Formation of most cell junctions, including gap junctions, tight junctions, and those involved in cell adhesion, such as spot desmosomes and adherent junctions, depends on an intimate plasma membrane interaction between adjacent cells mediated by cell adhesion molecules (8–11). To our knowledge, the last three classes of cell junctions have not been found at leukocyte–leukocyte or leukocyte–endothelial cell contacts. However, gap junctions, which are present between most cell types (12, 13), appear to occur *in vitro* between some activated white blood cells. For example, functional and morphological evidence of gap junctional communication between phytohemagglutinin-activated lymphocytes has been reported (14–16). In addition, dye coupling was reported between lymphocytes and endothelial cells (17).

Gap junctions are clusters of intercellular channels permeable to ions and small molecules that provide direct cytoplasmic communication between adjacent cells (12, 13). Each of these intercellular channels is a dodecamer assembly of protein subunits termed connexins, which are encoded by a gene family (18, 19). A gap junction channel is formed by two hemichannels or connexons, one contributed by each of two adjacent cells. In a not entirely consistent nomenclature, the junctions are termed homotypic if two connexons are made of the same connexin or if the cells are of the same type. The junctions are termed heterotypic if they are formed by different connexins in the two cells or if the cells are of different types. Hemichannels containing one connexin are homomeric; those with more than one connexin are heteromeric (20).

During the inflammatory response extensive leukocyte–leukocyte and leukocyte–endothelial cell contact occurs. Also, cultured vascular endothelial cells (21), a mouse macrophage cell line (J774) (22), and the macrophage foam cells of atherosclerotic lesions (23) express connexin 43 (Cx43) mRNA. These findings suggest that leukocytes could form homotypic gap junctions with each other and heterotypic gap junctions with endothelial cells at the site of inflammation provided that Cx43 (or another compatible connexin) is expressed during the acute response.

We report here that freshly isolated circulating leukocytes did not express Cx43 but that after lipopolysaccharide (LPS) treatment *in vitro*, Cx43 was clearly present. Furthermore, in two types of inflammatory response, LPS-induced peritoneal inflammation and that following ischemia–reperfusion of the cheek pouch, leukocytes expressed Cx43. In addition, leukocytes that adhered to the inner wall of venules after ischemia–reperfusion formed close appositions with endothelial cells.

MATERIALS AND METHODS

Reagents. LPS from *Escherichia coli*, phenylmethylsulfonyl fluoride, and fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG were obtained from Sigma. Formaldehyde, picric acid, acetic acid, ethanol, and other solvents and reagents were from Merck. A previously characterized rabbit polyclonal anti-Cx43 antibody was used (24, 25). This antibody was kindly provided by Elliot L. Hertzberg (Albert Einstein College of Medicine, Bronx, NY).

Animals. Male golden Syrian hamsters (100–120 g body weight) maintained with food and water ad libitum were obtained from the animal facilities of the Pontificia Universidad Católica de Chile.

Collection and Activation of Circulating Leukocytes. Animals were anesthetized with sodium pentobarbital (65 mg/kg, i.p.); blood was drawn from a cannula in the left carotid artery and received in glass microcapillaries with heparin as anticoagulant. After centrifugation in a hematocrit centrifuge, the white cells that lay on the red cells were carefully aspirated with a syringe, transferred to Eppendorf tubes, and washed once

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Abbreviations: Cx43, connexin 43; DAPI, 4′,6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; LPS, lipopolysaccharide.

with Hanks' balanced salts solution. Contaminating red blood cells were lysed by suspension in 155 mM NH_4Cl/2.7 mM $KHCO_3$/3.7 mM EDTA for 15 min at room temperature. Surviving white cells were then washed three times in Hanks' solution by centrifuging at 2000 rpm for 5 min in a 1.5-ml Eppendorf tube and resuspended. The purity of white cells in smears analyzed with Giemsa's stain was always >95%. The leukocytes were then incubated for 3 hr with either LPS at 1 μg/ml in Hanks' solution (LPS-treated) or the vehicle (Hanks' solution, control) at 37°C in 5% CO_2/95% air.

Collection of Peritoneal Leukocytes. Hamsters were injected intraperitoneally with 1 μg of LPS dissolved in 1 ml of Hanks' solution (LPS-treated) or the vehicle (control). After 5–7 days, the peritoneal cavity was washed with 5 ml of saline and drained with a 21-gauge needle under ether anesthesia. The cells contained in the peritoneal lavage were washed in Hanks' solution as described above and prepared for Western blot analysis or immunocytochemistry.

Ischemia–Reperfusion of the Cheek Pouch. Ischemia–reperfusion was induced in the check pouch as described by Yasuhara *et al.* (26). In brief, the hamster cheek pouch was prepared for intravital microscopy by inserting a Lucite plate and a fiber optic bundle through the mouth and placing a hollow Lucite chamber through an incision in the skin on the external surface of the pouch (27). A 1.7-cm-diameter cheek pouch area within the chamber was superfused with bicarbonate buffer, 1 ml/min, at 37°C, pH 7.35 (152 mM NaCl/4.7 mM KCl/1.17 mM $MgSO_4$/2.0 mM $CaCl_2$/20.0 mM $NaHCO_3$, equilibrated with 95% N_2/5% CO_2). The animal was placed under a Nikon Optiphot microscope connected to a television camera and a video display and recording system.

To occlude the circulation two chambers were constructed with walls of 0.2-mm-thick Mylar film. The walls were 3 mm high and circumscribed an equilateral triangular region 5 mm on a side. The top was covered with coverslip glass. Two regions of the exposed cheek pouch were circumscribed by the chambers. Ischemia was induced in one region by gently applying pressure to the coverslip with a micromanipulator until blood flow ceased. Reperfusion was allowed after 1 hr of ischemia by releasing the pressure, while microvascular flow and leukocyte rolling and/or sticking to the vascular wall were monitored on the television screen. The experiment ended after 1 hr of reperfusion, when the animal was killed with an excess of anesthesia, and the observed cheek pouch area was submitted to different fixation procedures for immunocytochemistry or electron microscopy. To analyze only leukocytes that had adhered to the endothelium *in vivo*, the tissue was washed of circulating blood by perfusing the animals through the aorta with 20 ml of bicarbonate buffer at ≈100 mmHg (1 mmHg = 133 Pa). Clearing of red blood cells in the postischemic and control areas was assessed by microscopy.

Immunoblots. Cells in suspension were centrifuged at 12,000 rpm in an Eppendorf microcentrifuge and then lysed by a 10-sec sonication (sonicator model W-225R; Heat Systems/Ultrasonics) in 100 μl of 2 mM phenylmethylsulfonyl fluoride containing 0.5 mg of leupeptin, 10 mg of soybean trypsin inhibitor, 100 mg of aminocaproic acid, at 4°C. Proteins were measured in aliquots of the cell lysates by the method of Bradford (28) with the Bio-Rad protein assay.

Eighty micrograms of protein was run in SDS/8% polyacrylamide vertical slab gels in a discontinuous buffer system as described by Laemmli (29) (Studier 16-cm slab unit; Hoefer) and proteins were electrotransferred to nitrocellulose. Immunoblot detection of Cx43 was performed with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate as color reagent for alkaline phosphatase (30).

Prestained low molecular weight markers and/or an aliquot of hamster heart homogenate was used to identify the electrophoretic mobility of Cx43.

Immunofluorescence. Smears of white cells on glass slides were fixed in 70% ethanol for 20 min at −20°C. They were stained for Cx43 by indirect immunofluorescence (FITC green emission, low-pass filter) as described below, photographed, and then subjected to nuclear staining with 4′,6-diamidino-2-phenylindole (DAPI) and photographed (UV excitation, blue emission filter). Alternatively, they were briefly stained with Giemsa stain, photographed, destained with 70% ethanol, washed with phosphate-buffered saline (PBS), and stained for Cx43, and then the fields taken previously were photographed for Cx43 through a fluorescence microscope.

Cheek pouch specimens were fixed with Bouin fixative placed on the surface of the working area. After 1 hr of fixation the area was excised and immersed in fixative for another 12 hr at room temperature. The tissue was dehydrated (ethanol, 50, 70, 80, 96, and 100%; followed by xylol, 100%) and mounted in paraffin (Paraplast), and 5-μm sections were obtained with a Spencer 820 microtome (American Optical). Fixed cells or tissue sections were incubated in blocking solution [5 mM EDTA/1% fish gelatin/0.05% Nonidet P-40/1% bovine serum albumin (essentially immunoglobulin free)/1% goat serum] for 30 min at room temperature. Then they were incubated with anti-Cx43 antibody (1:100 in blocking solution) overnight at 4°C. Samples were rinsed five times in Tris-buffered saline (pH 7.4) and then incubated with FITC-conjugated secondary antibody for 1 hr at room temperature, washed five times with PBS, and mounted under a coverslip with 1% *p*-phenylenediamine in 33% glycerol/PBS (10:1). Cx43 labeling was evaluated under a Nikon epifluorescent microscope equipped with a xenon arc lamp. Specificity of the immunoreactivity was assessed by replacing the primary antibody with preimmune serum or blocking solution.

Electron Microscopy. The cheek pouch was fixed by simultaneous intraarterial perfusion and topical application of 2.5% glutaraldehyde/4% formaldehyde/100 mM phosphate buffer, pH 7.4. After 10 min of fixation, pieces of about 1 mm^2 of the cheek pouch were cut and fixed for an additional 2 hr at 4°C. Pieces of tissue were postfixed with 2% OsO_4 for 2 hr under constant agitation. After dehydration with ethanol and acetone, samples were embedded in Epon Araldite [Pelco Medcast Kit; Ted Pella (Redding, CA)] for 72 hr at 60°C. Thin sections were made with an MT-2 Sorvall microtome, mounted

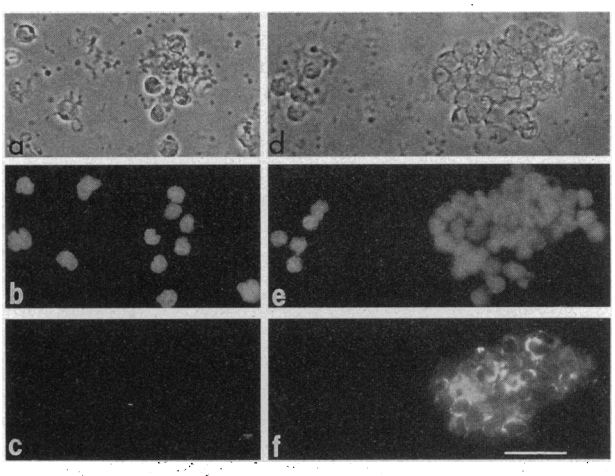

FIG. 1. Immunocytochemical detection of Cx43 in circulating leukocytes. White blood cells were incubated for 1 hr with Hanks' solution (*a–c*) or with LPS (*d–f*). Glass smears were stained for Cx43 (*c* and *f*) and then subjected to nuclear staining with DAPI (*b* and *e*). *a* and *d* are phase-contrast views. Fluorescence in control cells was never above background (*c*), while Cx43 labeling was observed in a high percentage of cells treated with LPS (*f*). Cx43 labeling could be weak or strong in the cytoplasm and was often localized at membrane appositions of LPS-treated cells. (Bar = 50 μm.)

on copper grids, counterstained with 4% uranyl acetate in methanol, and observed under a Phillips 102 transmission electron microscope.

RESULTS

Cx43 in Circulating Leukocytes. Smears of isolated control or LPS-treated white cells (Fig. 1 *a* and *d*) were first stained for Cx43 (Fig. 1 *c* and *f*) and then briefly stained with DAPI to visualize the nuclei (Fig. 1 *b* and *e*). Although control leukocytes were negative for Cx43 (Fig. 1*c*), many LPS-treated leukocytes showed prominent Cx43 immunostaining (Fig. 1*f*). Analysis of smears ($n = 12$) showed that 23.6 ± 4.1% (mean ± SEM) of LPS-treated leukocytes were immunoreactive for Cx43 and corresponded to polymorphonuclear cells and monocytes. LPS-treated lymphocytes were negative for Cx43 (data not shown).

In smears of leukocytes incubated for 3 hr in Hanks' solution, the number of clusters of three or more cells per field (×20 objective) was 0.86 ± 0.49. In smears of leukocytes incubated for 3 hr with LPS the number of clusters increased significantly to 3.2 ± 0.3 clusters per field ($P < 0.0001$ vs. control, *t* test). Leukocytes incubated for 1 hr with LPS showed a similar degree of white cell clustering (2.6 ± 0.3 clusters per field) as compared to 3 hr of LPS treatment.

Cx43 in Peritoneal Lavage Cells. Slightly more cells were recovered by lavage from the peritoneum of LPS-injected animals than from control animals (1.76 ± 0.25 vs. 1.39 ± 0.20 × 10^6 cells per ml of lavage; $P < 0.01$, $n = 3$). The percentage of Cx43-immunoreactive cells doubled from 23.2 ± 2.5% in control to 45 ± 3.1% in LPS-treated animals ($P < 0.0001$, $n = 12$). In controls most of the positive cells showed faint perinuclear labeling; <5% showed a diffuse labeling throughout the cytoplasm (Fig. 2*b*). In contrast, positive cells from LPS-treated animals showed strong Cx43 immunoreactivity (Fig. 2*d*) that in some clusters was prominent at membrane appositions (Fig. 2*e*).

Immunoblotting of 80 μg of protein from cells of control hamsters showed a barely detectable band (Fig. 2*f*, lane 1) corresponding to the unphosphorylated form of Cx43 detected in heart samples (lane 3). An equivalent amount of protein in cells from LPS-treated hamsters showed clearly detectable levels of the unphosphorylated form of Cx43 (Fig. 2*f*, lane 2). In contrast to heart samples, peritoneal white cells showed little if any of the phosphorylated forms of Cx43 (P1, P2, P3, and HP) described previously (ref. 25; Fig. 2*f*, lane 3).

Cx43 and Close Appositions in Adhering Leukocytes After Ischemia–Reperfusion. In nonischemic cheek pouch perfused for 5–10 min with saline through the aorta, few cells adhered to the inner wall of venules (Fig. 3*a*). In contrast, in areas submitted to ischemia–reperfusion many leukocytes became attached to the venular inner wall, frequently as clusters (Fig. 3*c*). The adherent cells after reperfusion stained for Cx43 (Fig. 3*d*), but Cx43 labeling was absent in normally perfused areas (Fig. 3*b*).

Thin sections of areas subjected to ischemia–reperfusion showed leukocytes in close contact with each other (data not shown) and with endothelial cells (Fig. 4*a*). Higher magnification of membrane appositions revealed small areas of close

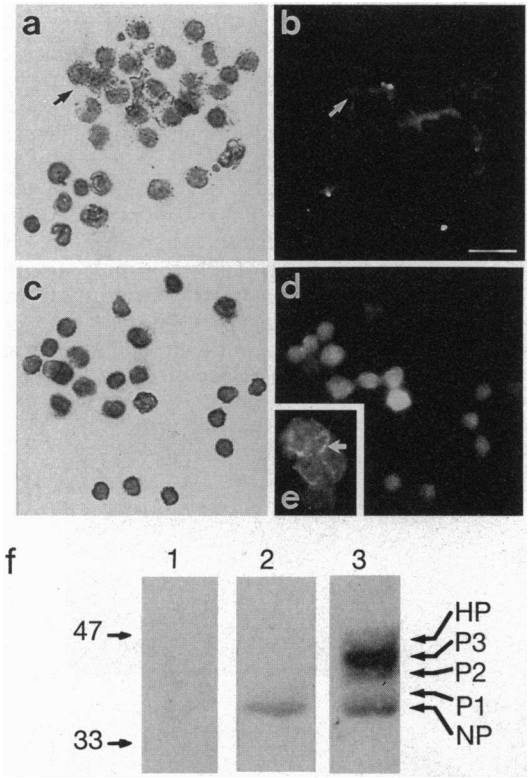

FIG. 2. (*a–e*) Immunocytochemical detection of Cx43 in cells obtained by peritoneal lavage. White cells were obtained from peritoneal lavage 5 days after intraperitoneal injection of Hanks' solution (*a* and *b*) or 1 μg of LPS (*c–e*). Equal dilutions of lavaged cells were stained with Giemsa reagent (*a* and *c*) and Cx43 antibody (*b*, *d*, and *e*). Control peritoneal cells often formed clusters and showed weak Cx43 labeling in perinuclear regions and along cell contacts (arrow in *b*). In comparison, Cx43 immunoreactivity was observed in a higher proportion of cells from LPS-treated animals, which also showed stronger signal labeling (*d*). In some clusters labeling was prominent at membrane appositions (arrow in *e*). (Bar = 20 μm in *a–d* and 40 μm in *e*). (*f*) Western blot analysis of Cx43. At left, the mobilities and sizes (kDa) of low-range molecular weight standards are indicated. Lane 1, protein (80 μg) of total homogenate from peritoneal cells of control; only a barely detectable Cx43 band is present. Lane 2, protein from LPS-treated hamsters; a Cx43 band is clearly present. Lane 3, hamster heart homogenate (50 μg of protein); multiple bands corresponding to phosphorylated (P1, P2, P3, and HP) and unphosphorylated (NP) forms of Cx43 are present as described previously (25).

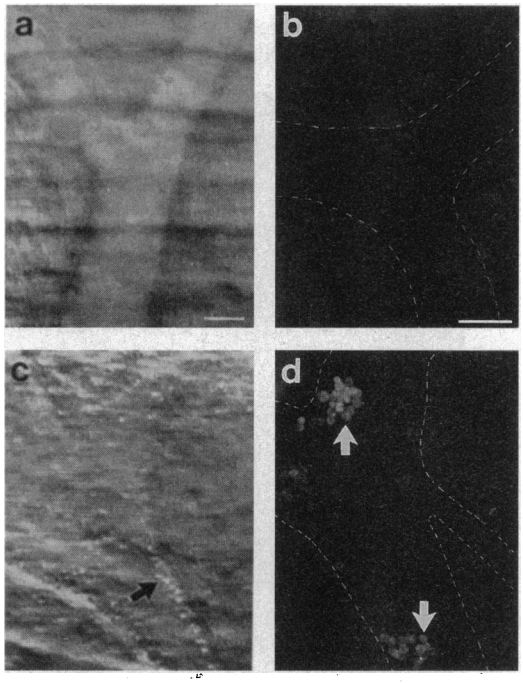

FIG. 3. Hamster cheek pouch microcirculation. Nonischemic control areas of the cheek pouch (*a* and *b*) or areas submitted to 1 hr of ischemia followed by 1 hr of reperfusion (*c* and *d*), as seen *in situ* from the television screen, after 10 min of saline washing through the aorta (*a* and *c*) or after fixation and immunostaining for Cx43 (*b* and *c*; different areas from *a* and *c*). A large number of adhering leukocytes are present in venules of postischemic tissue (*c*) but are not seen in control vessels (*a*). Immunofluorescence revealed strong Cx43 labeling (arrows) in adherent leukocytes of ischemic areas (*d*). Broken lines in *b* and *d* indicate vessel walls. (Bars = 40 μm.)

contact with dimensions similar to those characteristic of gap junctions (Fig. 4*b*).

DISCUSSION

Cx43 is expressed by a mouse macrophage cell line and by human macrophage foam cells but not by differentiated monocytes/macrophages or freshly isolated blood monocytes (22, 23). Consistently, monocytes present in our freshly isolated leukocyte preparations did not express Cx43. We extended this observation to hamster polymorphonuclear cells, which in freshly isolated leukocyte preparations are the most abundant leukocytes and were also devoid of Cx43. In addition, expression of Cx43 was induced by endotoxin (LPS) or by factors that are presumably released from tissue subjected to ischemia–reperfusion, two clinical conditions that lead to an acute inflammatory response. This finding might explain why freshly isolated and presumably nonactivated differentiated monocytes do not become dye-coupled with each other or with endothelial cells *in vitro* (23). Moreover, it could explain the presence of Cx43 mRNA detected by *in situ* hybridization in resident macrophages (foam cells) of human atherosclerotic carotid arteries (23), which may have been activated during migration to the atheromas.

In 1976, it was reported that murine macrophages maintained in primary culture for several days assume a linear orientation and develop a low-resistance pathway for transfer of electrical stimuli that can be detected across several cells (31). This finding, in conjunction with our observation that activated leukocytes form more clusters than unactivated cells and express Cx43, suggests that the low-resistance pathway between macrophages is mediated by gap junctions formed at least in part by Cx43.

Studies on leukocyte kinetics have shown that monocytes leaving the circulation in response to inflammation accumulate at sites of tissue injury more slowly than neutrophils. However, after 24 hr, monocytes predominate at sites of injury (32). The majority of the cells that we obtained from the peritoneum several days after endotoxin-induced inflammation were Cx43-positive and probably were monocytes since they did not show a polylobulated nucleus characteristic of granulocytes. In these cells, only a small fraction of the Cx43 was phosphorylated and presumably located at the plasma membrane; most were unphosphorylated and presumably corresponded to the diffuse intracellular labeling. In other cell types where unphosphorylated Cx43 has been found, the protein is predominantly located in a cytoplasmic compartment and is not forming functional gap junctions (10).

White blood cells adhere weakly to the endothelium and roll along the vessel wall at a rate ≈1% of the bulk flow rate of nonadherent red blood cells (33). It is now accepted that rolling is the consequence of weak adhesive interactions between the leukocytes and endothelium. This process may serve to detain the leukocytes near the inflammatory sites, enabling them to detect signals released from insulated tissues, which could lead to an acute or chronic inflammatory response. The orderly influx of white blood cells into an inflammatory site involves cell- and tissue-specific mechanisms of leukocyte–endothelial cell adhesion that induce rolling leukocytes to become "stickers" at the site of inflammation (33). This process involves a variety of intercellular molecules, including cytokines, chemokines, and nonproteinaceous agonists and their receptors (33). Various combinations of the diverse mediators permit the appropriate type of leukocytes to invade the correct body site at the opportune time, providing the organism with highly effective immunosurveillance. Consistent with previous reports (26, 34), we observed that within 1 hr of reperfusion after a period of ischemia, leukocytes firmly adhered to the endothelium of venules and remained attached even though the vessels were perfused with saline for 5–10 min. Thus there had been expression of adhesion molecules responsible for leukocytes becoming stickers (35). Synthesis of Cx43 had also occurred within the same period of time. Gap junction formation between leukocytes and endothelial cells may require intimate cell contact mediated by cell adhesion mole-

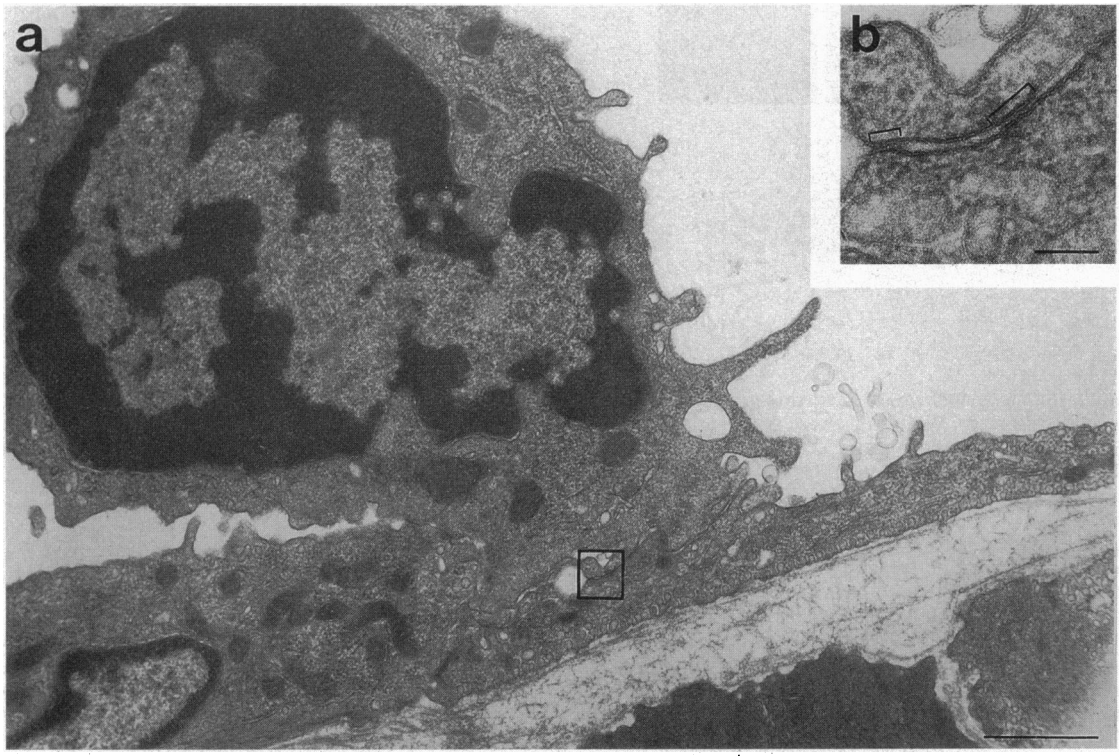

FIG. 4. Electron microscopy of leukocyte–endothelium interaction after ischemia–reperfusion. (*a*) A neutrophil adherent to the endothelium of a postcapillary venule ≈20 μm in diameter. The membranes of the two cells are closely apposed. (*b*) The area enclosed by a rectangle in *a*, enlarged 5.5 times. Lines indicate close appositions of membranes compatible with the presence of gap junctions. (Bars = 1 μm in *a* and 0.1 μm in *b*.)

cules, as shown for other cell types (8–11). In addition, adhesiveness may also be conferred by the interaction between the extracellular loops of connexons of contacting cells.

We identified small close membrane contacts likely to be gap junctions in electron micrographs of appositions between leukocytes and endothelial cells after reperfusion that were similar to those previously reported between cultured murine macrophages (32). The presence of gap junctions in this system is consistent with reports of dye coupling between blood lymphocytes and endothelium (17).

Although our data indicate that leukocytes can express Cx43, we do not know whether gap junctions potentially formed during their contact with endothelium are homotypic or heterotypic. Although Cx37 and Cx40 have been detected in the endothelium of small arteries (36, 37), to our knowledge the connexin type expressed by the endothelial cells of venules has not been identified. It is possible that they express Cx40 and Cx43 as do endothelial cells from human umbilical vein in primary culture (36, 38). Moreover, a mouse macrophage cell line (J774) expresses Cx43 mRNA but not the mRNAs of Cx26, -32, -37, or -46 (22, 36). In addition, the mRNA of Cx37 was reported to be absent from peripheral blood monocytes, monocyte-derived macrophages, and lymphocytes (36). Therefore, the only connexin type so far detected in activated polymorphonuclear cells and monocytes is Cx43. With paired *Xenopus* oocytes it has been shown that Cx43 and Cx40 are incompatible and do not form heterotypic channels but that Cx43 can form functional channels with Cx37 (37), suggesting that heterotypic junctions formed by those two proteins may contribute the gap junctions observed between leukocytes and endothelial cells after reperfusion.

The *in vitro* induction of Cx43 expression in white blood cells could have been the direct consequence of the interaction between these cells and LPS or of the autocrine effect of LPS-induced cytokines (39, 40). The induction of Cx43 expression observed *in vivo* after ischemia–reperfusion may have been triggered by inflammatory mediators and/or chemoattractants, including interleukins, cytokines, and platelet-activating factor released locally by activated blood cells and/or by activated endothelial cells (39, 41). Moreover, it has been reported that the addition of monocytes to human aortic endothelial cells cocultured with human aortic smooth muscle cells increases the levels of Cx43 mRNA (21). Although the cell type(s) showing the increase in Cx43 mRNA was not identified, factors secreted by the endothelial cells and aortic smooth muscle cells known to activate mononuclear phagocytes (42), such as the granulocyte/macrophage-colony-stimulating factor (21), might have mediated the increase.

Several cell types, including neutrophils, monocytes, eosinophils, basophils, and lymphocytes, are recruited to sites of inflammation and adhere to the endothelium. Other cells able to adhere to endothelial cells are the natural killer cells after activation with interleukin 2 (43). In addition, metastatic tumor cells can become dye coupled to cells of vascular endothelium in coculture (44), and metastasis-forming leukemia cells form gap junctions with myeloid sinus endothelium during the transmural passage of the leukemia cells (45). Therefore, gap junction formation between endothelial and migratory cells, including white blood cells and metastatic tumor cells, might be a general requirement for cells to cross the endothelium under physiological (diapedesis) and pathophysiological (inflammation, atherosclerosis, metastasis) conditions. Transfer of signals from the endothelium-adhered cell through these gap junctions might occur and induce destabilization and redistribution of tight junctions between endothelial cells that would allow for the extravasation of the migratory cell.

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1. Carlos, T. M. & Harlan, J. M. (1990) *Immunol. Rev.* **114,** 5–24.
2. Butcher, E. C. (1991) *Cell* **67,** 1033–1036.
3. Mayadas, T. N., Johnson, R. C., Rayburn, H., Hynes, R. O. & Wagner, D. D. (1993) *Cell* **74,** 541–554.
4. Bevilacqua, M. P., Pober, J. S., Mendrick, D. L., Cotran, R. S. & Gimbrone, M. A., Jr. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9238–9242.
5. Geng, J. G., Bevilacqua, M. P., Moore, K. L., Mcintyre, T. M., Prescott, S. M., Kim, J. M., Bliss, G. A., Zimmerman, G. A. & Mcever, R. P. (1990) *Nature (London)* **343,** 757–760.
6. Arnout, M. A. (1993) *Curr. Opin. Hematol.* **1,** 113–122.
7. Albelda, S. M. & Buck, C. A. (1990) *FASEB J.* **4,** 2868–2880.
8. Keane, R. W., Mehta, P. P., Rose, B., Honig, L. S. & Loewenstein, W. (1988) *J. Cell Biol.* **106,** 1307–1319.
9. Mege, R. M., Matsuzaki, F., Gallin, W. F., Golber, J. I., Cunningham, B. A. & Edelman, G. M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7274–7278.
10. Musil, L. S., Cunningham, B. C., Edelman, G. M. & Goodenough, D. A. (1990) *J. Cell Biol.* **111,** 2077–2088.
11. Jongen, W. M. F., Fitzgeral, D. J., Asamoto, M., Piccoli, C., Slaga, T. J., Gros, D., Takeichi, M. & Yamasaki, H. (1991) *J. Cell Biol.* **114,** 545–555.
12. Bennett, M. V. L., Barrio, L. C., Bargiello, T. A., Hertzberg, E. L., Spray, D. C. & Sáez, J. C. (1991) *Neuron* **6,** 305–320.
13. Dermietzel, R. & Spray, D. C. (1993) *Trends Neurosci.* **16,** 186–192.
14. Hülser, D. F. & Peters, J. H. (1971) *Eur. J. Immunol.* **1,** 494–495.
15. Hülser, D. F. & Peters, J. H. (1972) *Tissue Cell Res.* **74,** 319–326.
16. Oliveira-Castro, G. & Dos Reis, G. A. (1977) in *Intercellular Communication*, ed. De Mello, W. C. (Plenum, New York), pp. 201–230.
17. Guinan, E. C., Smith, B., Davies, P. F. & Pober, J. S. (1988) *Am. J. Pathol.* **132,** 406–409.
18. Beyer, E. C., Paul, D. L. & Goodenough, D. A. (1990) *J. Membr. Biol.* **116,** 187–194.
19. Willecke, K., Hennemann, H., Dahl, E., Jungbluth, S. & Heynkes, R. (1991) *Eur. J. Cell Biol.* **56,** 1–7.
20. Bennett, M. V. L. & Verselis, V. (1992) *Semin. Cell Biol.* **3,** 29–47.
21. Navab, M., Liao, F., Hough, G. P., Ross, L. A., Van Lenten, B. J., Rajavashisth, T. B., Lusis, A. J., Laks, H., Drinkwater, D. C. & Fogelman, A. M. (1991) *J. Clin. Invest.* **87,** 1763–1772.
22. Beyer, E. C. & Steinberg, T. H. (1991) *J. Biol. Chem.* **266,** 7971–7974.
23. Polacek, D., Lal, L., Volin, M. V. & Davies, P. F. (1993) *Am. J. Pathol.* **142,** 593–606.
24. Yamamoto, T., Ochalski, A., Hertzberg, E. L. & Nagy, J. I. (1990) *Brain Res.* **508,** 313–319.
25. Berthoud, V. M., Rook, M. B., Traub, O., Hertzberg, E. L. & Sáez, J. C. (1993) *Eur. J. Cell Biol.* **62,** 384–396.
26. Yasuhara, H., Hobson, R. W., II, Dillon, P. K. & Durán, W. N. (1991) *Am. J. Physiol.* **261,** H1626–H1629.
27. Boric, M. P., Donoso, M. V., Fournier, A., St. Pierre, S. & Huidobro-Toro, J. P. (1990) *Eur. J. Pharmacol.* **178,** 123–133.
28. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
29. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
30. Risley, M., Tan, I., Roy, C. & Sáez, J. C. (1992) *J. Cell Sci.* **103,** 81–96.
31. Levy, J. A., Weiss, R. M., Dirksen, E. R. & Rosen, M. R. (1976) *Exp. Cell Res.* **103,** 375–385.
32. Issekutz, T. B., Issekutz, A. C. & Movat, H. Z. (1981) *Am. J. Pathol.* **103,** 47–55.
33. Lasky, L. A. (1993) *Curr. Biol.* **3,** 680–682.
34. Granger, D. N., Benoit, J. N., Susuki, M. & Grisham, M. B. (1989) *Am. J. Physiol.* **257,** G683–G688.
35. Lefer, A. M. & Lefer, D. J. (1993) *Ann. Rev. Pharmacol. Toxicol.* **33,** 71–90.
36. Reed, K. E., Westphale, E. M., Larson, D. M., Wang, H.-Z., Veenstra, R. D. & Beyer, E. C. (1993) *J. Clin. Invest.* **91,** 997–1004.
37. Bruzzone, R., Haefliger, J.-A., Gimlich, R. L. & Paul, D. L. (1993) *Mol. Biol. Cell* **4,** 7–20.
38. Larson, D. M., Haudenschild, C. C. & Beyer, E. C. (1990) *Circ. Res.* **66,** 1074–1080.
39. Zimmerman, G. A. & Hill, H. R. (1984) *Thromb. Res.* **35,** 203–217.
40. Wing, E. J. (1993) in *Hemopoietic Growth Factors and Mononuclear Phagocytes*, ed. van Furth, R. (Karger, Basel), pp. 157–167.
41. Durán, W. N., Suval, W. D., Boric, M. P. & Hobson, R. W., II (1994) in *Vascular Surgery Principles and Practice*, eds. Veith, F. J., Hobson, R. W., II, Williams, R. A. & Wilson, S. E. (McGraw-Hill, New York), pp. 388–396.
42. Grabstein, K. H., Urdal, D. L., Tushinski, R. J., Mochizuki, D. Y., Price, V. L., Cantrell, M. A. & Gillis, S. (1986) *Science* **232,** 506–508.
43. Aronson, F. R., Libby, P., Bradon, E. P., Janicka, M. W. & Mier, J. W. (1988) *J. Immunol.* **141,** 158–163.
44. El-Sabban, M. E. & Pauli, B. U. (1991) *J. Cell Biol.* **115,** 1375–1382.
45. De Bruyn, P., Cho, Y. & Micelson, S. (1989) *Am. J. Anat.* **186,** 115–126.