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Gap junctions in cells of the immune system: structure, regulation and possible functional roles

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Abstract

Gap junction channels are sites of cytoplasmic communication between contacting cells. In vertebrates, they consist of protein subunits denoted connexins (Cxs) which are encoded by a gene family. According to their Cx composition, gap junction channels show different gating and permeability properties that define which ions and small molecules permeate them. Differences in Cx primary sequences suggest that channels composed of different Cxs are regulated differentially by intracellular pathways under specific physiological conditions. Functional roles of gap junction channels could be defined by the relative importance of permeant substances, resulting in coordination of electrical and/or metabolic cellular responses. Cells of the native and specific immune systems establish transient homo- and heterocellular contacts at various steps of the immune response. Morphological and functional studies reported during the last three decades have revealed that many intercellular contacts between cells in the immune response present gap junctions or "gap junction-like" structures. Partial characterization of the molecular composition of some of these plasma membrane structures and regulatory mechanisms that control them have been published recently. Studies designed to elucidate their physiological roles suggest that they might permit coordination of cellular events which favor the effective and timely response of the immune system.

Key words
- Cell contacts
- Gap junctions
- Connexins
- Native immune response
- Specific immune response
- Inflammatory response

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Introduction

Numerous reports have described different mechanisms for intercellular communication between members of the immune system, including cell adhesion molecules, membrane molecules that act as ligand-receptors (1) and soluble molecules secreted into the extracellular milieu which act as paracrine and autocrine signals (2). In contrast, little attention has been given to gap junction intercellular communication. Recently, two reviews have described gap junction communication between cells of the immune system (3,4). The present article attempts to provide an updated and brief review on gap junctions expressed by different cellular members of native and specific immune systems.

Gap junctions have recently been reviewed (5). Briefly, gap junction channels span the plasma membrane of two adjacent

cells and each cell contributes one half of the channel, called connexon. Each hemichannel consists of the oligomers of six protein subunits termed connexins (Cxs). A gene family of which at least 14 different homologous members have been identified in rodents encodes these proteins. Cxs are named according to their molecular mass predicted from their cloned DNA sequence. Most cells, excluding vertebrate skeletal muscle, red blood cells and spermatozoids, form gap junctions and express two or more Cx types. Cells of invertebrate organisms express functionally equivalent channels, but their protein subunits are not members of the Cx family (6). In recent reports, it has been shown that gap junction channels and hemichannels can result from the interaction of two different Cxs (5). Moreover, numerous reports have shown that gap junction communication can be regulated at various cellular levels, including mRNA transcription, mRNA stability and channel gating (5). Gap junction channels formed by different Cx types present different gating and permeability properties that fulfill different functions and thus are adjusted to the various regulatory mechanisms present in each cell type and under different physiological conditions.

The first descriptions of electrical coupling between activated lymphocytes were reported in the early 1970's (7-9). Further evidence of gap junctional communication, including transfer of fluorescent dyes, metabolic cooperativity and electron microscopy studies (thin sections and freeze fracture replicas), reported at that time have been recently reviewed (3,4). The homocellular gap junctional communication at cell-cell contacts between other members of the immune system, such as macrophages (10,11), follicular dendritic cells (12), thymic epithelial cells (13), polymorphonuclear (PMN) cells (14,15) and microglia (16) has also been reported. In addition, it has been shown that heterocellular contacts between macrophages and neutrophils (17), and leukemia cells and myeloid sinus endothelium (18) contain gap junctions. Similarly, "gap junction-like" structures as heterocellular contacts between Langerhans cells and T-cells (4,19-21), lymphocytes and endothelial cells (22) and PMN cells and endothelial cells (14) have been described. Moreover, heterocellular gap junctional communication between lymphocytes and endothelial cells (23), thymocytes and thymic epithelial cells (13), macrophages and epithelial cells (24-26), mastocytoma cells and lymphocytes (27) and follicular dendritic cells and B-cells (28) has also been reported.

Gap junctions in the bone marrow and secondary lymphoid organs

Both *in vivo* (29) and *in vitro* (30,31) studies have demonstrated that bone marrow stromal cells form gap junctions. Morphological and functional studies have also shown gap junctions between bone marrow stromal cells as well as between stromal and hematopoietic progenitor cells (28,32-35). In primary cultures of bone marrow, the extent of dye transfer between cells increases progressively with time in culture (35), suggesting that *in vitro* cells are free from the environmental factors present *in vivo* that restrain the functional expression of gap junction. Consistently, the reduction in hematopoietic tissue induced with fluorouracil is followed by a dramatic increase in the number of gap junctions between bone marrow cells and the number falls back to the normal values before the bone is filled with marrow (36).

Stromal cells do not express Cxs 26 and 32 and communicate with each other through gap junctions that contain Cx43 (31,37). It is not known whether stromal cells and hematopoietic progenitor cells express other Cxs, as described for most cells of other systems (5). Treatment with interleukin-1 or TNF-α (37), but not irradiation (30), reduces

gap junctional communication between stromal cells. Similarly, differentiation of stromal cells to adipocytes is associated with a reduction in gap junctional communication (30) and Cx43 reactivity (31). Gap junctions are more abundant in hematopoietic stem cells before growth (36) and in cells of different types of leukemias that present an increased stromal:hematopoietic cell ratio (37). Megakaryocytes present in normal bone marrow contain Cx43, but not Cx26 or Cx32 (38). During migration, gap junction-like structures have been identified between neutrophils or lymphocytes and cells of the sinusoidal wall (adventitial or endothelial cells) of the bone marrow (39).

The first report of gap junction formation between follicular dendritic cells showed that these cells express Cx43, but not Cx32 or Cx26 (12). Recently, this analysis has been extended showing that they also contain Cx37 (4). In mouse lymph nodes, both Cxs 37 and 43, but not Cxs 26, 32, 33, 45 or 50, are present in follicular dendritic cells, interdigitating cells, T-cells and B-cells (4,40). At least the reactivity to Cx43 in follicular dendritic cells is inducible. These Cxs are frequently detected at cell-cell contacts, suggesting that they form functional channels. In agreement, dye transfer between cultured human dendritic cells and B lymphocytes has been shown (28). Gap junctions found at heterocellular contacts might be relevant in the diverse events of the immune response that occur within lymph nodes, including antigen presentation and lymphocyte proliferation.

Carolan and Pitts (41) have shown metabolic coupling between thymocytes, suggesting that they establish gap junctional communication. This possibility was recently supported by the demonstration of electrical coupling and dye transfer between these cells and blockade of intercellular communication with octanol, a conventional gap junction blocker (13). In addition, thymic epithelial cells and thymocytes communicate with each other through gap junctions that contain at least Cx43 (13). In cultured thymic epithelial cells a significant amount of Cx43 is phosphorylated and cells are well coupled (13).

Gap junctions in the native immune system

The main cell components of the native immune system are cell barriers (endothelia and epithelia), granulocytes, monocytes/macrophages, and natural killer cells. All endothelial and epithelial cells studied express Cxs. Both cell types frequently retain Cx expression and gap junction communication in primary cultures (Figure 1). Exposure to inflammatory mediators reduces gap junction communication between cultured endothelial cells. TNF-α and interleukin-1 reduce dye coupling between human umbilical vein endothelial cells (HUVECs) (42,43). The effect of TNF-α on the expression of Cxs by HUVEC is differential; while Cxs 37 and 40 are reduced, Cx43 remains unchanged (43). Moreover, histamine reduces gap junction communication between high vascular endothelial cells isolated from human tonsils (Figure 1). In myoendothelial preparations treated with lipopolysaccharides (LPS), TNF-α, or IL-1ß, homocellular coupling remains unchanged but the heterocellular coupling is drastically reduced (44). Similarly, the heterocellular coupling between rat brain endothelial cells and astrocytes is transiently reduced by TNF-α (45).

Ultrastructural and functional evidence indicates that migratory leukocytes found at inflammatory foci form gap junction-like structures with the endothelial cells of the microcirculation. After ischemia-reperfusion (14) or during the initial stage of autoimmune demyelinization (22), specific subsets of circulating leukocytes (neutrophils and lymphocytes, respectively) form "gap junction-like" structures with the endothelium. Moreover, bidirectional dye (calcein) trans-

fer between lymphocytes and endothelial cells (23) or macrophages P388D1 and IEC-6 epithelial cells has been demonstrated (24-26). In the latter system, gap junction-dependent propagation of Ca^{2+} waves in response to mechanical stimulation has also been shown (25), suggesting that these two cell types perform coordinated activities and/or one regulates the state of the other through a Ca^{2+}-dependent mechanism mediated by gap junctions. Polarity of dye movement has been found in studies of gap junction permeability between smooth muscle and endothelial cells of hamster cheek pouch arterioles (46), suggesting the existence of a directional preference for diffusion of intercellular signals and/or metabolites. It is not known whether gap junctions formed between leukocytes and cellular barriers show unidirectional permeability preferences.

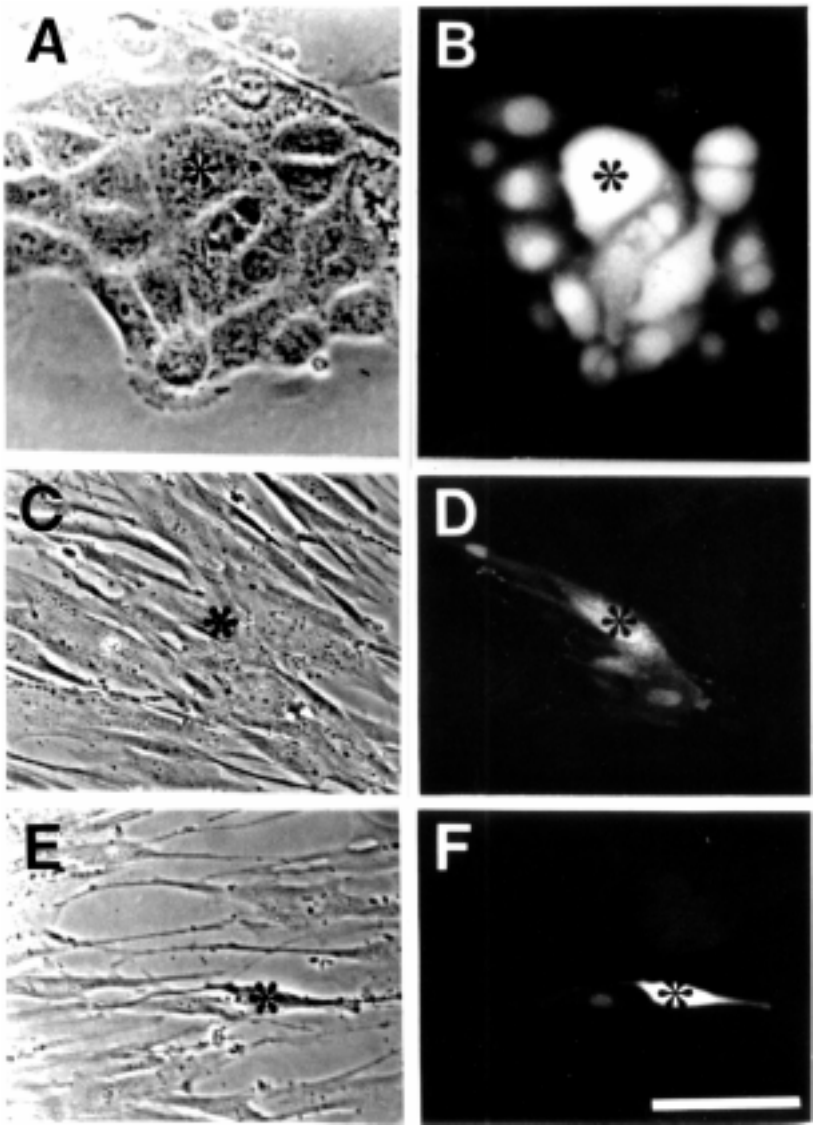

Figure 1 - Dye coupling between epithelial or endothelial cells. Dye coupling tested by microinjecting Lucifer yellow-CH into one cell and observing its spreading to adjacent cells is found in a subconfluent culture of MDCK cells (B) and a confluent culture of high vascular endothelial cells isolated from human tonsils (D). Dye coupling is drastically reduced (F) in high vascular endothelial cells treated for 1 h with 0.1 µM histamine. A, C and E are phase contrast views of the fluorescent fields shown in B, D and F, respectively. Bar: 100 µm.

In vertebrates, the main blood cell members of the native immune response are PMN cells of which the most abundant are neutrophils. Available information indicates that the expression of Cxs in these cells is inducible. Activated human PMN cells form homocellular gap junctions *in vitro* (15). Moreover, circulating hamster leukocytes do not express Cx43 and after incubation with LPS for 1 h they become reactive to anti-Cx43 antibodies (Figure 2) (14), suggesting that the expression of this protein is inducible. In addition, the application of platelet activating factor (PAF) to the hamster cheek pouch induces recruitment and firm adhesion of Cx43 positive PMN cells to the endothelium of the microcirculation, but fails to induce the expression of Cx43 in isolated leukocytes (47), indicating that PAF-induced Cx43 expression observed *in vivo* might not result from the direct PAF-hamster leukocyte interaction. Similarly, LPS induces formation of human PMN aggregates and translocation of Cx43 towards the plasma membrane, but cells remain dye uncoupled. Nevertheless, LPS-activated PMN cells in medium conditioned by rat brain endothelial cells treated with LPS develop prominent dye coupling (15).

Depending on the circulatory region, endothelial cells express Cx43 and Cx40 and/or Cx37 (5). Since these Cxs form gap junctions with different permeability and gating properties (5), differences in Cx composition of the homocellular (endothelial cell-endothelial cell) and heterocellular (endo-

thelial cell-smooth muscle cell) gap junctions formed might explain the dye movement polarity found in hamster cheek pouch arterioles (46). During an inflammatory response, endothelial cells also form gap junctions with activated leukocytes (14), suggesting that endothelial Cxs are sorted to the apical membrane to form gap junction channels with compatible leukocyte Cxs.

Connective tissues contain a variety of cells with defense and immune functions, such as tissue macrophages and mast cells. The first demonstrations of gap junctional communication between cultured canine and murine macrophage cells were reported two decades ago (10,11). But, it was only during the last decade that Cx43 was detected in several macrophage types, including the murine cell line J774 (48), macrophage foam cells from arteriosclerotic lesions (49), peritoneal macrophages (14), kidney macrophages in inflammatory renal disease (50), Kupffer cells (51), microglia (16) and Langerhans cells (4). Cx43 mRNA has been detected in cultured monocytes/macrophages (52), but not in freshly isolated human monocytes/macrophages (49). Moreover, it has been recently reported that mast cells express Cxs 32 and 43, but not Cx26 (53).

J774 macrophages (54), human monocytes/macrophages or HUVECs and monocytes/macrophages (49) do not establish intercellular communication in culture. Nevertheless, P388D1 or J744 macrophages cocultured with epithelial cell lines show homocellular dye coupling, as well as heterocellular dye coupling with epithelial cell lines (25), suggesting that soluble factors present in the co-culture induce macrophages to form gap junctions. In support of this possibility, culture medium conditioned with endothelial cells derived from rat brain microcirculation induces dye coupling (Figure 3) and translocation of Cxs from the cytoplasmic compartment to the plasma membrane in J774 cells (Eugenín EA, Garcés G and Sáez JC, unpublished observation). Microglia, the main immune effector of the central nervous system, also become dye coupled when cultured for a few hours in medium conditioned by rat brain endothelial cells (Eugenín EA, Martínez AD and Sáez JC, unpublished observation). Dye coupling between microglia is also observed after 4-9-h treatment with a calcium ionophore (16) (Figure 3), suggesting that activated macrophages can establish gap junctional communication.

Structural and functional studies have demonstrated cell junctions equivalent to gap junctions between invertebrate blood cells (hemocytes) (55). These cells establish functional intercellular communication within seconds when they are pushed together (55), suggesting that hemocytes present a preformed pool of hemichannels for

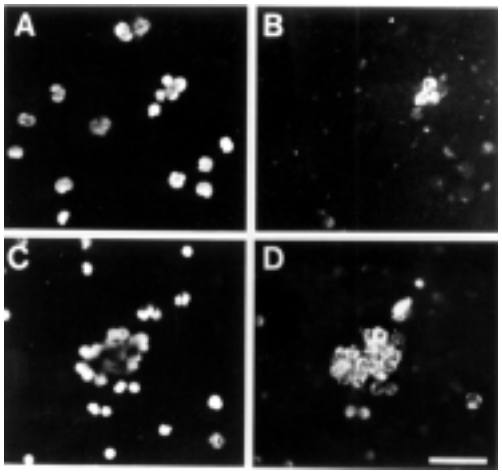

Figure 2 - Cx43 is not found in circulating PMN cells and its expression is induced by LPS. Most freshly isolated hamster leukocytes incubated for 3 h at 37ºC in culture medium containing 5% FBS remained as singlet cells and very few were immunoreactive to Cx43 (B). Nonetheless, cells treated with 1 mg/ml LPS for 3 h formed many aggregates and were immunoreactive to Cx43 (D). In each situation, the cells shown in (B) and (D) were identified by their nuclear staining with DAPI in A and C, respectively. Bar: 75 µm.

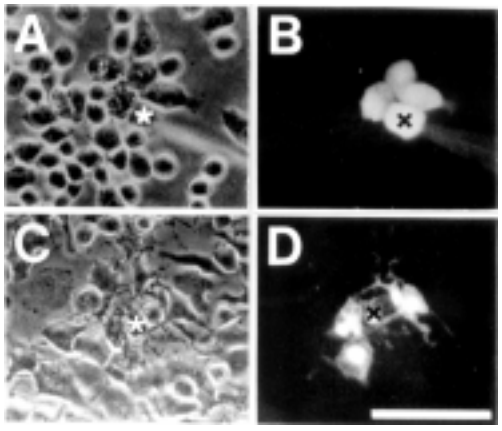

Figure 3 - Induction of dye coupling between macrophage cells. In cultures of (B) rat microglia treated for 3 h with the calcium ionophore 8Br-A23187 (2 µM) or (D) murine macrophages (J774) treated for 3 h with medium conditioned for 24 h by rat brain endothelial cells there was dye transfer to several neighboring cells. A and D are phase contrast views of the fluorescent fields shown in B and D, respectively. Bar: 120 µm.

ready formation of intercellular channels. The structural components of these channels remain unknown, but it is likely that they are proteins homologous to those described to form intercellular channels in *Drosophila melanogaster* and *C. elegans*, termed innexins (6).

Gap junctions between cells of the specific immune system

Activation of a specific immune response requires a direct physical interaction between antigen-presenting cells and T-cells, the main cellular effector of the specific immune system (1). At Langerhans and T-cell interphases, gap junction-like structures have been identified both *in vitro* (19,20) and *in vivo* (21). At cell-cell contacts between cultured Langerhans cells and T-cells, at least Cx43 is detected (4). The formation of gap junction channels requires a cell-cell proximity mediated by cell adhesion molecules (5). Thus, the anti-vascular cell adhesion molecule-1 (VCAM-1) antibody-induced inhibition of the lymphocyte proliferative response in the allogeneic mixed lymphocyte reaction (56) might be the consequence, at least in part, of the blockade of a gap junction-dependent mechanism.

Lymphocytes (T-cells plus B-cells or just T-cells) treated with either concanavalin A (Con-A) or phytohemagglutinin (PHA) form clusters of variable sizes. Circulating human or bovine lymphocytes treated with PHA express a low resistance pathway that allows the intercellular transfer of electrical stimuli (7,8). Moreover, intercellular transfer of fluorescein or radiolabeled uridine has been found between mouse spleen lymphocytes, rabbit mesenteric lymphocytes, murine thymic lymphocytes and lymph node lymphocytes (4,27,41,57). Electrical coupling between activated lymphocytes is blocked by an increase in intracellular Ca^{2+} concentration (9). In addition, dye coupling is reversibly blocked with octanol and prevented with synthetic peptides homologous to the extracellular loop 1 of Cxs (40), supporting the idea that electrical and metabolic coupling between activated lymphocytes occurs through gap junction channels. Consistently, mouse lymphocytes contain Cxs 37 and 43, but not Cxs 32, 33, 40 or 50, and upon treatment with Con-A both Cxs are translocated from the plasma membrane to cellular interphases (40). The latter event occurs without changes in Cx levels, suggesting that freshly isolated lymph node lymphocytes contain a preformed pool of Cxs. On the other hand, *in vivo* studies have shown that Cx43 expression by cells of mouse lymph nodes is induced by the administration of antigen (28). Moreover, *in situ* hybridization studies have shown that follicular dendritic cells and lymphocytes of germinal centers of other secondary lymphoid organs, such as human tonsil and spleen, also express Cx43 (28).

Functional roles of gap junctions in cells of the immune system

Although in some systems reduced gap junction communication is associated with an increase in tissue function, such as amylase secretion by the exocrine pancreas, more frequently it has been demonstrated to cause tissue disfunction (5). Inhibition of gap junctional communication of the rat gastric mucosa in combination with ischemia-reperfusion weakens the barrier function of the gastric mucosa and causes damage to the barrier function (58). Moreover, in long-term cultures of bone marrow the blockade of gap junctions with amphotericin retards stem cell growth (37). In addition, blockade of thymocyte gap junctions with octanol reduces the secretion of thymulin (13).

Antigen presentation leads to T-cell activation and proliferation, responses of lymphocytes that are cell-cell contact-dependent (1,59), suggesting the involvement of cell-cell adhesion and/or gap junctional com-

munication. The latter possibility was recently supported by the finding that synthetic peptides homologous to the extracellular loop 1 of Cxs prevent gap junction formation and drastically reduce the DNA replication of Con-A-treated mouse lymphocytes (40). Thus, gap junctional communication between proliferating lymphocytes might coordinate their metabolic and cytokine-induced responses to allow the appropriate timing of the specific immune response. Similarly, the blockade of leukemic cell differentiation has been associated with their intercellular coupling to stromal cells (34).

The innate and specific immune responses involve homo- and heterocellular contacts essential for their normal functioning. In many of those events, gap junctional communication is established, but their functional roles remain speculative except for few cases described above for which direct or indirect evidence has been provided. A putative gap junction role is synchronization of cellular events during the transmigration across cellular barriers. Supporting this view, gap junctions have been observed between metastase-forming leukemia cells and myeloid sinus endothelium (18), polymorphonuclear and endothelial cells (14) and macrophages and epithelial cells (24-26).

Clearly, further studies are needed to understand the role of gap junctions in different physiological and pathophysiological functions of the immune system.

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