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Chapter 2

Gap junction-mediated intercellular communication in the immune system.

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Review

Gap junction-mediated intercellular communication in the immune system

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Abstract

Immune cells are usually considered non-attached blood cells, which would exclude the formation of gap junctions. This is a misconception since many immune cells express connexin 43 (Cx43) and other connexins and are often residing in tissue. The role of gap junctions is largely ignored by immunologists as is the immune system in the field of gap junction research. Here, the current knowledge of the distribution of connexins and the function of gap junctions in the immune system is discussed. Gap junctions appear to play many roles in antibody productions and specific immune responses and may be important in sensing danger in tissue by the immune system. Gap junctions not only transfer electrical and metabolic but also immunological information in the form of peptides for a process called cross-presentation. This is essential for proper immune responses to viruses and possibly tumours. Until now only 40 research papers on gap junctions in the immune system appeared and this will almost certainly expand with the increased mutual interest between the fields of immunology and gap junction research.

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Keywords: Gap junctions; Immune system; Cross-presentation; MHC class I molecules; Peptides; Immunological coupling

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Abbreviation: Cx, connexin; LPS, lipopolysaccharide; IFN- γ , interferon-gamma; NK cell, natural killer cell; CTL, cytotoxic T cell; MHC, major histocompatibility complex

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1. Introduction

Every modern household is nowadays connected to the outside world via an internet connection (the world-wide web), much like cells of a multicellular organism. Many cells can electronically communicate with their surrounding through small channels called gap junctions rather than fibreglass cables. These channels were first observed back in the 1950s by electrophysiological experiments in crayfish cells (Furshpan and Potter, 1959). It took a decade to realize that electrical signals were transferred via small channels connecting the cytosol of adjacent cells as visualized by electron microscopy (Revel and Karnovsky, 1967). These channels can facilitate the transfer of small molecules like ions, metabolites, second messengers and peptides up to 1.8 kDa (Nicholson, 2003; Neijssen et al., 2005). These different molecules that can be transferred via gap junctions allow electric, metabolic and immunological transfer of information and can direct processes like development, inflammation, cell death, immune responses but also muscle contractions via electrical coupling of cells. Although the function of gap junctions in heart muscle contraction (Rohr, 2004), hearing and other processes (Rabionet et al., 2002) is studied in detail, their function in the immune system is largely ignored.

Gap junctional coupling of adjacent cells occurs in every multicellular organism, but vertebrate and invertebrate organisms use different proteins for their construction. In invertebrate organisms like *Drosophila* and *Caenorhabditis elegans*, gap junctions are composed of proteins from the innexin family (Phelan, 2005). In vertebrates more than 20 isoforms of connexins have been identified. They are classified on their molecular mass that varies between 25 and 62 kDa. In humans the family of connexins comprises 20 members (Willecke et al., 2002).

A gap junction is a channel connecting the cytoplasms of two adjacent cells. A functional channel is formed when an assembly of six connexin molecules—called connexon—is transported to the plasma membrane and docks to a similar connexon in the opposing membrane of a neighbouring cell (Unger et al., 1999) (Fig. 1A). All connexin molecules have four membrane spanning domains, two extracellular domains and a large cytoplasmic C-terminal tail that has an important role in the gating of the channel (Fig. 1B). They are look-alikes of the very large family of tetraspanin proteins with ~30 members (Hemler, 2005). The gating of the channels, or the opening and closing can be controlled post-translationally by various growth factors (Lampe and Lau, 2004). For example, the C-terminal tail of Cx43 contains several motifs that can be subject of regulation by protein kinases. The phosphorylation of the tail of Cx43, and thus the gating of the channel, is

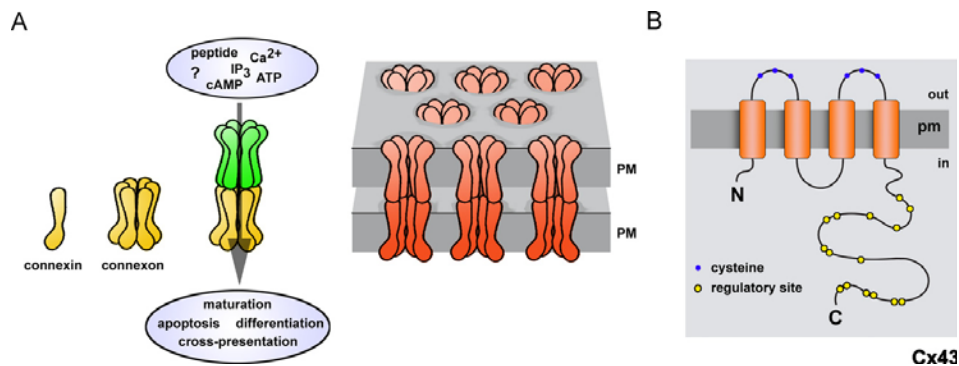


Fig. 1. Structural organization of Cx43 and the assembly of gap junctions. (A) A gap junction is an assembly of six connexin molecules, comprising a hemi-channel called connexon that forms a functional channel when it is connected to a hemi-channel present in the membrane of a neighbouring cell. These channels facilitate passive diffusion of small molecules and peptides involved in a number of processes like cell maturation and differentiation, apoptotic cell death and cross-presentation. (B) Connexin molecules span the plasma membrane (pm) 4 times, which exposes two extracellular loops to the extracellular space. In both extracellular loops three conserved cysteine residues (blue dots) are present, which are critical for the docking of a hemi-channel to a hemi-channel in the membrane of an adjacent cell. The large C-terminal cytoplasmic tail harbours several regulatory sites for phosphorylation (yellow dots) that control the gating of the channel.

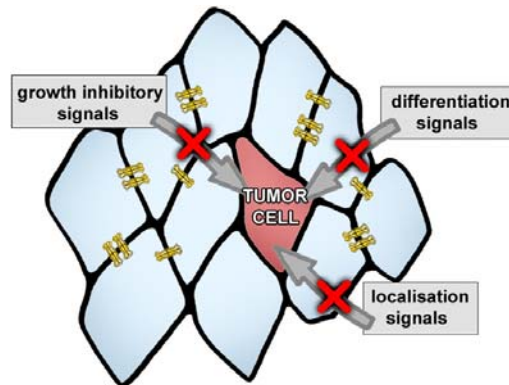


Fig. 2. Autarkic lifestyle of tumours as a result of downregulation of gap junctions. Most tumour cells downregulate the expression of connexin to uncouple their cytosol from that of surrounding cells via gap junctions. As a result they will not receive growth inhibitory signals or differentiation stop signals. The autarkic tumour cells also do not communicate with surrounding cells.

carried out via kinases that can be downstream of the G protein-coupled receptor signalling pathways (Fig. 1B). The structure and regulation of gap junctions is reviewed in more detail in [Nicholson \(2003\)](#) and [Lampe and Lau \(2004\)](#). Most tumour cells are deficient in gap junctions and re-introduction of gap junctions may result in a growth deficit of these transformed cells ([Krutovskikh et al., 2000](#); [Trosko et al., 2004](#)). The absence of gap junctions in these tumours enables the tumour to live autarkic because growth inhibitory, differentiation and localization signals cannot be delivered (Fig. 2).

The size permeability for small molecules and the connected electrophysiological properties of a gap junction are dependent on the gap junction pore size as generated by the respective connexin isoforms ([Weber et al., 2004](#)). Since multiple connexin isotypes can be expressed in the same cells, gap junctions composed of different connexin isotypes could be constructed. The different connexin isoforms show high sequence homology in the transmembrane and extracellular regions, especially the three conserved cysteine residues involved in the docking of two opposing hemi-channels (Fig. 1B). The main differences between the various isotypes can be found in the intracellular C-terminal tail, which may explain the differences in regulation and selectivity of these channels.

2. Tissue distribution and functions of connexins

Whereas most connexin isoforms are expressed in a strictly tissue-specific manner, one isotype—Cx43—is expressed ubiquitously. Cx43 was first characterized in heart muscle cells ([Shibata and Yamamoto, 1977](#)). Gap junctions constructed of Cx43 electrically couple heart muscle cells and control synchronous heart muscle contractions. Cx43-deficient mice show a plethora of defects and die postnatally as the result of ventricular arrhythmia ([Reaume et al., 1995](#)). Other gap junctions are more restrictively expressed and their malfunctioning results in different disorders like deafness, skin diseases, fertility problems and lens cataracts (reviewed in [Nicholson, 2003](#)).

In principle, all cells expressing connexins can form functional gap junctions with their direct neighbours as long as they express connexins as well. These interactions are called homotypic, when both cells express the same connexons, or heterotypic when two different connexons are connected ([Weber et al., 2004](#)). Both homo- and heterotypic interactions are observed in multicellular organisms. Many cell types express multiple isotypes of connexin and this may allow formation of heterotypic channels. It has been shown that connexons composed of different connexins (Cx26 with 32 and Cx40 with 43) can result in functional channels ([Elfgang et al., 1995](#); [He et al., 1999](#)), but it is unknown whether this is generally correct for every heterotypic channel. Since two connexon hemi-channels have to interact at the interphase of two adjacent cells, tight cell-to-cell

contacts (especially found in 3D tissues) will increase the probability to form a functional gap junction. Hemi-channels were believed to be non-functional without pairing to their neighbouring counterpart and ultimately destroyed by the proteasome. However, recent evidence indicates that hemi-channels can be expressed at the plasma membrane for transfer of molecules (ATP) from the cytosol to the extracellular medium. Cx30 hemi-channels that allowed transfer of ATP in the extracellular medium were found in cells of patients with hidrotic ectodermal dysplasia (HED), resulting in altered proliferation and differentiation of keratinocytes. HED patients have mutations in their *GJB6* gene that encodes for Cx30, and these mutations might prevent rapid internalization of hemi-channels (Essenfelder et al., 2004). The pathogen *Shigella flexneri* was shown to induce opening of Cx26 hemi-channels allowing the release of ATP, which might promote bacterial invasion and spreading (Tran Van Nhieu et al., 2003). Release of ATP from Cx37 hemi-channels in primary monocytes and macrophages was shown to inhibit monocyte adhesion, preventing the initiation and development of atherosclerotic plaques (Wong et al., 2006). However, it is unknown how these hemi-channels are stabilized at the plasma membrane and whether they act autonomously.

3. Gap junctions in cells of the immune system

Gap junctions are largely ignored by immunologists. This stems from the fact that most immunological experiments are performed *ex vivo* with non-adherent cells. Communication then follows the secretion of soluble factors—like interleukins—in the extracellular environment and recognition of the ligand by transmembrane receptors on the acceptor cell. Still a restricted set of connexins (Cx43 and more rarely Cx40, Cx37, Cx32, Cx30.3) are expressed in many immune and haematopoietic cells (Oviedo-Orta and Howard Evans (2004)) (Table 1). How their expression levels compare those of other cells is unknown. Some immune cells, like human monocytes, express Cx43 after encountering signals for infections like LPS and IFN- γ (Eugenin et al., 2003) but this has not been studied in further detail, and the immunological consequences of Cx43 upregulation following ‘infection signals’ are unclear. Connexins can make functional gap junctions between immune cells but also other cells like endothelial (Jara et al., 1995; Krenacs and Rosendaal, 1995; Oviedo-Orta et al., 2000; Polacek et al., 1993) and stromal cells (Krenacs and Rosendaal, 1998; Ploemacher et al., 2000). Since immune cells are usually motile (they can migrate to other locations like lymph nodes after having visited tissues), they can transfer intracellular information obtained after gap junction contact with residing tissue cells to other locations in an organism.

Multiple cell types of the immune system have been shown to express connexins. These cells usually have different functions, and control immune responses or execute these in a series of different manners (for review see Oviedo-Orta et al., 2001). For example, phagocytic cells of the innate immune system are dendritic cells (DCs) and monocytes/macrophages. These cells express connexins and can form functional gap junctions between identical (Eugenin et al., 2003; Matsue et al., 2006) as well as different cells (Krenacs et al., 1997; Martin et al., 1998). Most notably, connexins are expressed by almost all immune cells and can be upregulated when the immune cells become exposed to inflammatory factors (Eugenin et al., 2003). Thus, immune cells can communicate via gap junctions but a function has only been resolved in particular unrelated cases, which will be discussed below (Fig. 3A).

(i) Immune cells originate in the bone marrow. Immune stem cells located in the bone marrow may communicate with their surrounding stromal cells since both express Cx43 (Cancelas et al., 2000). These stem cells can differentiate into the nine defined circulating blood cells, including all members of the cellular immune system. In this early phases of haematopoiesis, Cx43-containing gap junctions appear to be critical for terminal differentiation of primary T and B cells as tested in Cx43-deficient mice (Montecino-Rodriguez et al., 2000).

(ii) T and B cells further develop or are activated in lymphoid organs like the thymus and the lymph nodes. In the thymus, progenitor T cells have extensive contact with the surrounding thymic epithelial cells and thymic DCs. These cell types do express Cx43, possibly allowing homo- and heterotypic interactions. Cx43 is required for correct T cell maturation as shown in Cx43-deficient mice (by unknown signals). (Montecino-Rodriguez et al., 2000). In secondary lymphoid organs (usually lymph nodes) T cells encounter antigen presented by DCs. T cells that are not deleted during negative selection in the thymus migrate into peripheral tissues to search for antigenic information in the form of peptides. The expression of Cx43 by these T cells

Table 1
Connexin expression in cells on the immune system

Connexin isotype	Cell types	Function	Coupled cell types	References
Cx43	Bone marrow derived DC	Cross-presentation DC activation	bmDC–tissue cells bmDC–bmDC	Neijssen et al. (2005) Matsue et al. (2006)
	Bone marrow stromal cells	Haematopoiesis	Bone marrow stromal cells–HSCs	Cancelas et al. (2000)
	Haematopoietic stem cells			
	Follicular DC	Germinal centre development	FDC–B cells	Krenacs et al. (1997)
	Monocytes	Cross-presentation	FDC–FDC Monocytes–tissue cells	Eugenin et al. (2003) and Neijssen et al. (2005)
	Tonsil DC Appendix DC		DCs–tissue cells Langerhans cells–tissue cells	
	Langerhans cells Macrophages	Inflammation	Macrophages–intestinal endothelium	Martin et al. (1998)
	Thymus epithelial cells	T cell development	TEC–T cell progenitor cells	Alves et al. (1995) and Fonseca et al. (2004)
	T cell progenitors			
	T cells	Maturation (?)	T cells–T cells	Oviedo-Orta et al. (2000)
	B cells	Antibody secretion	T cells–B cells	Oviedo-Orta et al. (2001)
	NK cells	Activation (?)	NK cells–NK cells	Oviedo-Orta et al. (2000)
	Mast cells	Unknown	Mast cells–mast cells Mast cells–fibroblasts	Vliagoftis et al. (1999)
Cx40	Polymorphonuclear neutrophils (PMN)	Extravasation	PMN–PMN PMN–endothelium	Jara et al. (1995) Zahler et al. (2003)
	T cells	Maturation (?)	T cells–T cells	Oviedo-Orta et al. (2002)
	B cells	Immunoglobulin secretion	T cells–B cells B cells–B cells	
Cx37	Macrophages	Atherogenesis	Macrophages–smooth muscle cells	Kwak et al. (2002)
	Monocytes	Prevention of atherosclerosis	Hemi-channel	Wong et al. (2006)
Cx32	Mast cells	Unknown	Mast cells–mast cells	Vliagoftis et al. (1999)
			Mast cells–surrounding cells	
Cx30.3	Thymocytes	Unknown	–	Fonseca et al. (2004)

Of the 20 known human isoforms of connexin (Cx), at least five are expressed in immune cells. Cx43 is expressed in almost all immune cells. In most cases expression of Cx can facilitate the formation of both homo- and heterotypic gap junctions. Transfer of information in the form of small molecules will play an important role in many processes critical for the proper functioning of the immune system.

might allow communication with surrounding tissues, possibly allowing proper activation/differentiation, but this is speculation at this point.

(iii) B cells also express Cx43. B cells may interact via gap junctions with follicular DCs in the secondary lymphoid organs. This coupling of these cells and B cells might help synchronizing germinal centre events or facilitate transfer of antiapoptotic molecules to rescue B cells from apoptosis (Krenacs et al., 1997). T, B and NK cells isolated from tonsils (also secondary lymphoid organs) express low levels of Cx40 as well, but the function of gap junctions in these cells and their connected tissue is unknown. It has been proposed that these

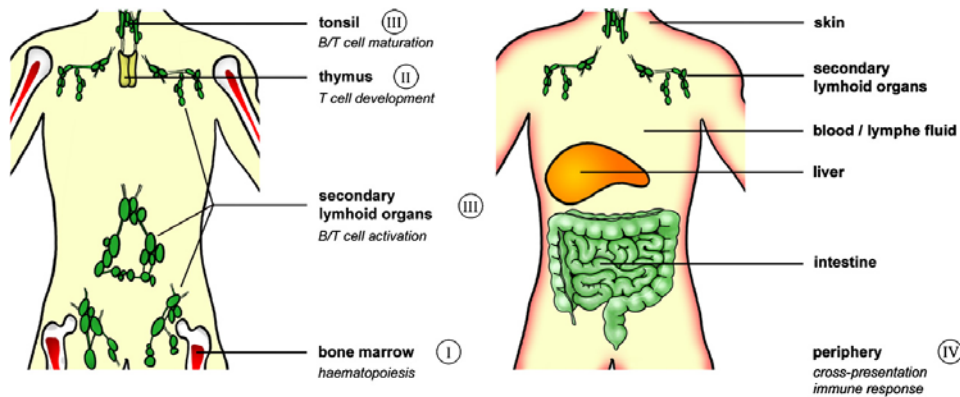


Fig. 3. Gap junctions in cells of the immune system. Immune cells can communicate via gap junctions but a function has only been resolved in particular cases. (i) Haematopoietic stem cells and their surrounding stromal cells express Cx43 and the coupling of these cells is essential for proper haematopoiesis. (ii) In the thymus T cells interact with the surrounding thymic epithelial cells via gap junctions and these interactions may be important in T cell development. (iii) In secondary lymphoid organs, such as the tonsil and lymph nodes, B and T cells might receive their further instructions from dendritic cells before they are released into the periphery. Gap junction-mediated interactions might allow the synchronization of these processes. (iv) Immune cells in the periphery, which comprise several tissue-specific types of dendritic cells, continuously sample the antigenic content of tissue cells. The dendritic cells can form temporal gap junctional connections to allow exchange of antigenic peptides (immunological coupling).

cells form hemi-channels composed of Cx40 and that these channels facilitate ATP-mediated propagation of calcium waves, but this is speculative (Oviedo-Orta et al., 2001, 2002). B cells ultimately produce antibodies and Thelper cells control this process. Oviedo-Orta et al. studied the role of gap junctions in a minimal system (mixed lymphocyte culture) and showed that gap junction communication is required for efficient antibody secretion by B cells (Oviedo-Orta et al., 2002). Which intracellular signals are exchanged is unclear but this result shows that gap junction-mediated intercellular signalling to B cells is required for optimal antibody production.

(iv) The ‘master regulators’ of the immune system (the DCs) also express gap junctions to communicate with their environment. In addition, human monocytes as well as DCs upregulate Cx43 and form gap junctions when detecting inflammation suggesting that they contact the environment to sample metabolic or electronic information from the neighbouring cells in response to infection (Eugenin et al., 2003; Neijssen et al., 2005). Many tissues express different sets of different members of the DC family (Fig. 3B), like liver DCs, DCs in the skin epithelial layer and intestinal DCs, which may communicate with the environment. But which information? Follicular DCs express Cx43 and may form functional contacts with the B cells in the tonsil (Krenacs et al., 1997). Furthermore, it has been shown recently that gap junction communication between DCs is required for their activation, but—again—it is unclear which signals are transferred between the cells (Matsue et al., 2006).

Immune cells can sample the electronic or metabolic information by gap junction communication, like most tissue cells. But can immune cells also detect the antigenic state of tissue and acquire this information for stimulation of the immune system?

4. Gap junction-mediated immunological coupling: antigen presentation

Gap junctions allow intercellular transfer of small (<1 kDa) ions and metabolites (Nicholson, 2003) and the question is whether information of pathogens also fulfils this criterion. In fact, the major form of viral antigen as presented by the immune system could fit this, because MHC class I molecules present fragments in the form of peptides of around only 9 amino acids (~1 kDa) to cytotoxic T cells (CTLs). These CTLs can then

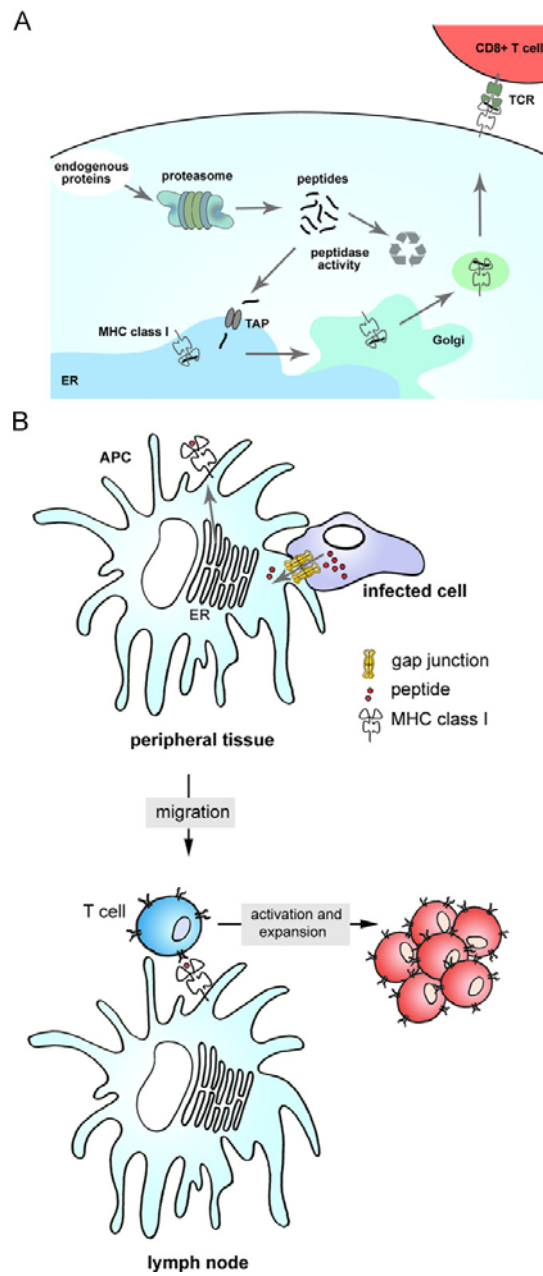
respond by killing an infected cell. Importantly, the majority of the peptides are derived from proteins degraded in the cytosol and a rather complicated series of biochemical steps have to be performed before a fragment of an intracellular protein can be presented in the form of a peptide by MHC class I molecules. This antigen processing and presentation pathway goes as follows (Fig. 4A; for review see Yewdell et al., 2003). Cytosolic proteins are degraded by the major intracellular protease, the proteasome. Most of the resulting peptides will be degraded by intracellular peptidases but some peptides escape this fate by binding to a dedicated ER-located peptide transporter called TAP. TAP pumps the peptides over the membrane of the ER and into the lumen where MHC class I molecules are retained until an appropriate peptide binds. Peptide binding to MHC class I molecules is required for final and correct folding and allows release of the peptide-loaded MHC class I molecule from the ER and transport to the plasma membrane for presentation to the immune system. This complicated system ensures that antigenic peptides are presented only by the cells that also express the protein, and not by the neighbouring cell, because peptides usually do not diffuse over membranes.

This obviously assumes that peptides cannot use gap junctions for cytosol-to-cytosol transfer to neighbouring cells. However, it was shown that peptides up to 1850 Da (or about 16 amino acids) could diffuse over gap junctions, which is considerably larger than the bulk of peptides presented by MHC class I molecules (~9 amino acids in size). These peptides are relatively stretched when presented in the context of MHC class I molecules (Madden et al., 1993) but probably present in many flexible conformations in solution. This discrepancy in cut-off size of gap junctions with the existing dogma of 1 kDa is most likely caused by the lack of secondary structure of peptides. Immunological relevant peptides (in the range of 8–14 amino acids) can be considered as flexible linear strands that can diffuse freely through gap junctions, in contrast to small molecules that have a 3D structure. This has been illustrated by comparing Cx43-mediated intercellular diffusion of a ‘linear’ versus a circular 8-mer peptide of identical sequence. Only the linear peptide diffused to other cells indicating that 3D conformation rather than molecular weight determines gap junctional passage (Neijssen et al., 2005). Hence, gap junctions connect the antigen processing machineries of two neighbouring cells allowing immunological coupling. As a result, innocent cells can acquire peptides from infected neighbours for presentation by MHC class I molecules, and CTL can then respond by not only killing the infected but also the direct non-infected neighbouring cell (Neijssen et al., 2005). Although this may appear an unnecessary waste when uninfected neighbours are eliminated by CTLs, in fact it is not. At least, when considering that the first cells to be infected by a virus are the direct neighbours, who can acquire antigenic peptides by gap junctional transfer even before they are producing intracellular proteins following infection. Of note, presentation of antigenic peptides from an infected cell is restricted to only their direct neighbour(s) by the cytosolic peptidases that destroy most peptides, also in the peptide-accepting cells (Reits et al., 2003). Immunological coupling by gap junction-mediated peptide transfer allows antigen presentation by other cells than the infected cells and is a new function for gap junctions.

5. Other roles for gap junction-mediated immunological coupling: cross-presentation

It does not suffice for (infected) tissue cell to present a viral peptide by its own MHC class I molecules to expect an efficient immune response. The immune system has to be instructed properly to initiate the activation and expansion of specific cytotoxic T lymphocytes. Professional antigen presenting cells (APCs), including DCs and macrophages, are responsible for this important task. APCs (Shen and Rock, 2006) have to acquire information from infected cells in the periphery and then transfer it to lymph nodes to specifically stimulate CTL for activation and expansion by presenting the antigen and expression of specific costimulatory molecules (Randolph et al., 2005; Sumen et al., 2004). Therefore, the APCs have to present in the context of their MHC class I molecules peptides generated in other (i.e. infected) cells. Transfer of antigenic information should occur in the immune system in a process called cross-presentation (Fig. 4b) (for in-depth review see Shen and Rock, 2006). Can gap junctions be used to transfer this immunological information from infected to DCs?

Activated monocytes (that could be precursors of the DCs) as well as DCs do express Cx43 and can form functional gap junctions with other cells (Eugenin et al., 2003; Matsue et al., 2006). In fact, activated monocytes are able to acquire antigenic information in the form of peptides from influenza-infected cells, but



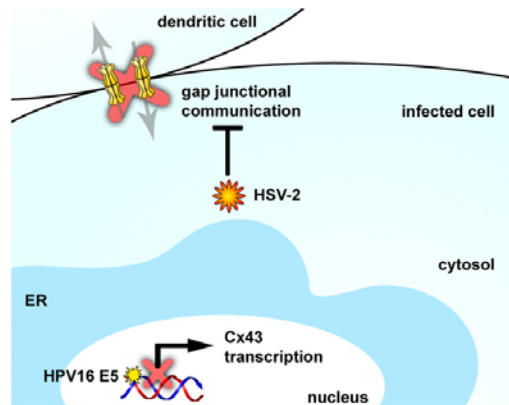


Fig. 5. Viral immune evasion by targeting gap junctions. Many viruses express proteins that interfere with direct MHC class I presentation or cross-presentation to prevent recognition of infected cells by the immune system. Some viruses like HPV16 and HSV-2 target gap junctions either at the transcriptional or post-translational level, possibly to prevent gap junction-mediated cross-presentation and thus activation of the immune system.

only after gap junctional contact (Neijssen et al., 2005). These gap junctions then allow immunological coupling by allowing cytosol-to-cytosol transfer of antigenic peptides for cross-presentation by activated monocytes and DCs. Tissue DCs then sense electric, metabolic and immunological alterations in their tissue surroundings and may respond by activation, by migrating to lymph nodes and by cross-presentation of antigenic information acquired in the periphery through gap junctional communication. The MHC class I molecules, that present peptides on the surface of cells, provide the immune system a sample of the protein content of a cell. Viruses express several proteins that inhibit the MHC class I presentation pathway, thus also preventing cross-presentation (Fig. 5) (Lilley and Ploegh, 2005). Herpes Simplex virus 2 (HSV-2) (Fischer et al., 2001) and Human Papilloma virus 16 (HPV16). (Oelze et al., 1995) express proteins that block gap junctional contact, possibly preventing cross-presentation, which will reduce the chance of being discovered by the immune system.

6. Other points

Because gap junctions allow electric, metabolic and immunological exchange of information, it comes as no surprise that gap junctions will have multiple functions in the immune system. Only in few cases, the molecules exchanged by gap junctions causing biological effects are defined.

Gap junctions can transfer various molecules and thus initiate different cell responses resulting in processes as variable as apoptosis, proliferation, migration and differentiation (Krenacs and Rosendaal, 1998; Ploemacher et al., 2000). As mentioned above, DCs can acquire antigenic information in the periphery by gap junction-mediated transfer of peptides. Since DCs have to transfer this information to lymph nodes for

Fig. 4. Antigen presentation; MHC class I antigen pathway and cross-presentation. (A) The MHC class I antigen presentation pathway starts with the degradation of endogenous (but also viral) proteins. The resulting peptides are further trimmed by cytosolic peptidases and the resulting amino acids recycled for the synthesis of new proteins. A small number of peptides escape degradation and is transported into the ER by TAP. In the ER, peptides can bind MHC class I molecules, provided that the peptide has the correct length and anchor residues. The MHC class I/peptide complex is then transported via the golgi to the plasma membrane for consideration by CTLs. (B) For the initiation of a proper immune response, CTLs need to be primed first by dendritic cells. These cells can sample the antigenic content of tissue cells by making temporal contacts via gap junctions that allow diffusion of peptides from the donor tissue cell to the acceptor dendritic cell and vice versa. MHC class I molecules thus present a peptide produced in a neighbouring cell, and this process is called cross-presentation.

stimulation of CTL expansion, additional signals are required to leave the tissue. These may also require gap junctional communication. For example, glioblastoma cells require Cx43 for expression of the chemokine CCL2 thus attracting CTL, monocytes and other immune cells (Huang et al., 2002). It may even be possible that danger signals in the form of small intracellular molecules move through gap junctions to deliver other signals to DCs to initiate migration, just as (unknown) apoptotic signals can be transferred (Krutovskikh et al., 2002). The ability of cells to propagate death signals via gap junctions was first observed in gene therapy experiments of Freeman et al. (1993). They observed massive cell death throughout a tumour even when only a fraction expressed a construct rendered them susceptible to cell death after administration of a drug. This phenomenon was called 'bystander death', as untransfected bystanders that would normally not be affected by the drug were killed. Subsequent reports showed a critical role for gap junctional communication for this 'bystander effect', as uncoupled cells or cells treated with gap junction inhibitors did not die in this experimental set-up (Cirenei et al., 1998; Dilber et al., 1997; Sanson et al., 2002). Dying cells are until late stages of apoptosis coupled via gap junctions to healthy neighbouring cells (Wilson et al., 2000). It was therefore hypothesized that cell fate modulators can be propagated via gap junctions. Kalvelyte et al. showed that introduction of Cx43 in cells that lack endogenous gap junctions increases the rate of apoptotic progression and that this could be inhibited by gap junctional inhibitors (Kalvelyte et al., 2003). The coupling of dying and healthy cells may also allow diffusion of ions and other substances from healthy to injured cells, thereby contributing to cell survival. Although it is not fully understood which signal(s) are involved in these important processes, it has been suggested that among others H_2O_2 , Ca^{2+} , IP_3 , ATP and cAMP can diffuse via gap junctions to neighbouring cells. In addition, metabolites specifically generated in dying cells, like uric acid (Mattson et al., 1997), or apoptotic metabolites that are activated during apoptosis could be transferred for bystander death (reviewed in more detail in Krysko et al., 2005). Paradoxically, cells neighbouring an infected cell may be eliminated by CTL after gap junction-mediated transfer of antigenic peptides (Neijssen et al., 2005), but also following gap junction-mediated transfer of apoptosis signals from the infected cell following the 'kiss of death' delivered by a CTL. This elimination of innocent bystander cells could provide a *cordon sanitaire* surrounding infected cells and kill these cells while they are at the earliest phases of infection or not yet infected. As mentioned above some viruses abrogate gap junctional communication of cells that are infected suggesting that recognition and clearance of these infected cells is delayed or prevented (Fischer et al., 2001; Oelze et al., 1995).

Gap junction communication is largely ignored by the immunological society. However, given the importance of this intercellular communication system in other biological processes, many surprises on the role of gap junction communication in the immune system can be awaited.

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