

Gap junction-mediated antigen transport in immune responses

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Communication between cells is a crucial part of the immune response. The importance of cytokines and immunological synapses for this purpose has long been recognized. Connexin-based gap junctions that allow exchange of molecules between adjacent cells also seem to have an important role. Recent work suggests that gap junction-mediated antigen transport might be a mechanism of immune-response regulation. Here, we discuss this idea in more detail and suggest possible ways in which this mechanism might have both positive and negative impacts during an immune response.

Introduction

A successful immune response requires communication between many different cell types. This communication occurs through soluble signaling proteins, such as chemokines or cytokines [1], by the formation of immunological synapses between cells [2] and the sharing of cell-surface proteins [3]. Another form of communication between cells relies on the formation of connexin-based gap junctions. Connexins are members of a diverse family of proteins that are differentially produced by many cell types [4,5]. Gap junctions are formed when two connexin-based hemichannels couple to form a channel between two cells, facilitating transport of small molecules (with sizes up to ~1kDa) [6,7].

Gap junctions have key roles in tissue homeostasis, inflammation and repair [8,9], hematopoiesis [10], cardiovascular function [11], and neuronal activity [12]. There is also evidence that gap junction dysregulation contributes to carcinogenesis [13,14], brain damage [15] and several other human diseases [16].

Several studies have indicated the involvement of gap junctions in the communication between cells of the immune system [17–24]. Indirect evidence for the importance of gap junctions in immunity comes from the observation that viruses – such as herpes simplex virus (HSV) and human papillomavirus (HPV) – actively influence gap junction communication [25–28]. Compounds known to be involved in immune responses – such as peptidoglycan, lipopolysaccharide (LPS), tumor necrosis factors (TNF) and interferons (IFN) – can influence the expression of gap junctions [29–34]. In addition, gap junction production has been shown to influence the production of cytokines and immunoglobulins [35].

Despite this evidence, little is known about the mechanisms by which gap junctions influence immune responses. However, an exception is a recent elegant *in vitro* study showing that gap junctions facilitate transport of antigen epitopes between cells, and these transported epitopes are subsequently presented on MHC I and can be recognized by cytotoxic T lymphocytes (CTL) [36]. It has been suggested that this process of gap junction-mediated antigen transport (GMAT) might be useful *in vivo* by leading to activation of antigen-presenting cells (APC) or reduction of viral load [36–39]. Here, we consider different scenarios in which GMAT might have an important role during virus infection, and we outline the circumstances under which we might expect the infected host to benefit from such a mechanism. In doing so, we also identify situations in which GMAT might be detrimental and could lead to increased viral load and immunopathology. Note that GMAT was demonstrated for gap junctions formed by coupling two connexin Cx43 hemichannels [36]. Although Cx43 is the most ubiquitous connexin in immune cells [24], it is possible that GMAT can occur via gap junctions formed by other connexins. In this article, we simply refer to gap junctions and GMAT in a general form. We return to this point at the end of the paper.

GMAT during priming of antigen-presenting cells

We begin by considering GMAT between infected cells and professional APC. Professional APC, such as dendritic cells (DCs), are crucial in the early stages of an immune response [40,41]. An activated, antigen-presenting DC can migrate to a draining lymph node where it activates CTL and T helper cells. To stimulate CTL, the DC needs to present antigen on MHC class I. Generally, antigen presented on MHC class I is produced within a cell (the endogenous presentation pathway), which enables the cell to signal to the outside that it is infected. DCs not infected by virus can also present antigen obtained from extracellular sources on MHC class I, in a process termed cross-presentation [42,43]. Several pathways for cross-presentation are known. For example, DCs can acquire antigen from the debris of dead cells, through sampling of live cellular material or through uptake of fragments of free virus [41,44]. Figure 1 illustrates these different cross-presentation pathways. Recently, it was found that gap junctions are important for DC activation [34], and it has been proposed that GMAT might assist cross-presentation [36].

GMAT between an infected cell and a DC could potentially increase the rate at which the DC acquires

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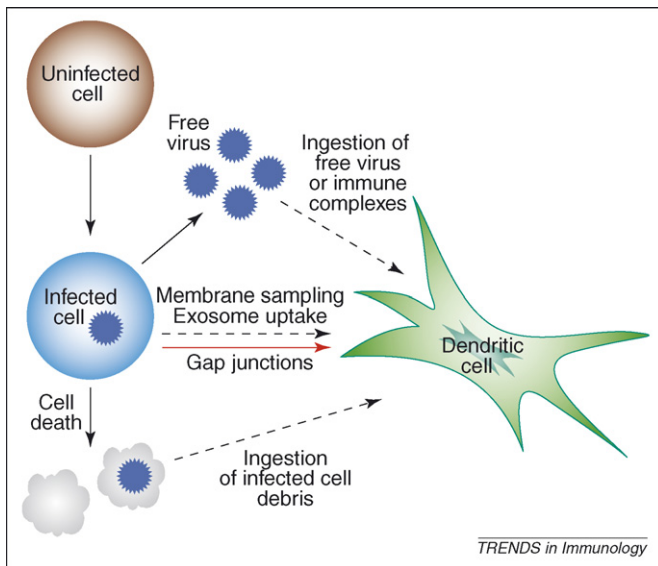


Figure 1. Schematic of antigen uptake for cross-presentation by a dendritic cell. The dendritic cell can obtain antigen by uptake of free virus fragments, by sampling the membranes of live infected cells, or by ingesting dead cell material that includes viral epitopes (black dashed arrows). In addition, viral antigen might enter the dendritic cell via gap junctions (red arrow).

antigen. Faster uptake of antigen would lead to faster accumulation of activated DCs, provided that appropriate activation stimuli ('danger' signals) occur simultaneously. The potential benefit of GMAT would probably depend on the type of virus. If the virus causes significant cell death and large amounts of new virus are produced rapidly, DCs can obtain antigen through uptake of debris or free viral proteins, and GMAT, therefore, might not contribute significantly to the activation process. Alternatively, if the virus is not cytolytic or kills cells slowly, or if the rate of release of new free virions is low, GMAT might influence DC activation significantly.

Suppression of GMAT might therefore be an important strategy for latent viruses. Presumably, during latency, little new virus is produced and latently infected cells do not die at an increased rate. If viral antigens are produced during latency (e.g. latency-associated transcripts in HSV) [45,46], the infected cells could transport antigen to nearby DCs via gap junctions. If antigen transfer is accompanied by a (possibly unrelated) danger signal, an immune response against the virus could be mounted. Therefore, transcription during latency of factors that cause downregulation of gap junctions might help to minimize the risk of detection. Although it is not known if such a mechanism would help latent viruses to evade immunosurveillance, it is interesting to note that HSV and HPV both undergo latency and have both been shown to downregulate gap junctions [25–28]. However, because many details of the latent phase and overall life cycle of both viruses are still not well-understood, the significance of this gap junction downregulation remains speculative.

Although it is unknown if GMAT occurs during DC activation *in vivo*, it seems that it would be beneficial for the immune system and help to speed the priming of DCs. But one can also think of a possible negative effect. If GMAT can occur between an infected cell and a DC, it is also probable that it can occur between two DCs, potentially

leading to distribution of antigen among DCs. Although this might be beneficial at high antigen concentrations and further accelerate the priming of DCs, at low antigen concentrations it is feasible that sharing of antigen leads to antigen dilution, resulting in the inability of DCs to activate CTL. This could be prevented if DCs downregulate gap junction expression after being activated and thereby prevent the loss of their antigen, similar to the downregulation of endocytosis observed for activated DCs [47].

GMAT between target cells

We now consider GMAT between infected and uninfected target cells. This process can lead to cells that display antigen on MHC class I without harboring virus. These uninfected, but antigen-presenting, bystander cells can become infected and thereby convert to a regular infected cell. Alternatively, activated CTL might recognize the viral antigen presented on the uninfected bystander cell and subsequently kill it. Infected cells are also killed by CTL and, additionally, die due to virus-induced mortality. Figure 2 schematically shows the different processes.

Removal of target cells

Increased GMAT can lead to increased presentation of antigen on uninfected bystander cells. This will probably result in increased killing of these antigen-presenting, but uninfected, bystander cells by CTL. The removal of potential future 'virus factories' (infected, virus-producing cells) might lead to a reduction in viral load, which in turn leads to reduced virus-induced cell death. However, it is also possible that this is more than offset by the increased killing of bystander cells, thereby increasing overall immunopathology. From this viewpoint, GMAT is only beneficial in situations in which the increase in cell death owing to bystander killing is less than the reduction of virus-induced cell death. The potential benefit of GMAT in this situation might, therefore, be biggest for viruses that produce a large number of progeny per infected cell.

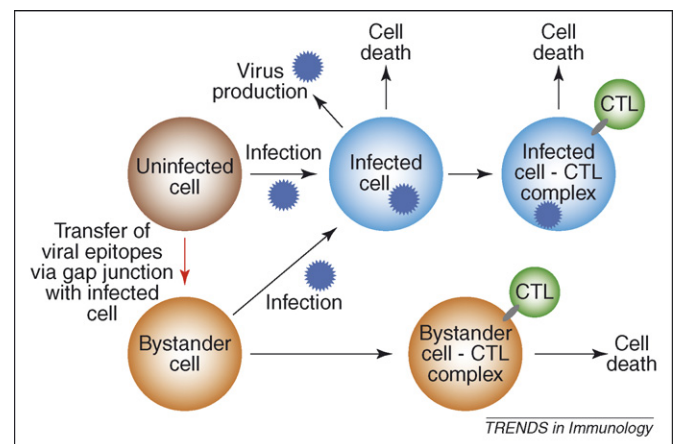


Figure 2. Schematic of gap junction-mediated antigen transfer (GMAT) between target cells. Uninfected cells become infected. Infected cells produce virus, and they also transfer viral antigen to neighboring uninfected cells via gap junctions and convert them into antigen-presenting, uninfected bystander cells. Cytotoxic T lymphocytes (CTL) can recognize antigen on both infected and bystander cells and kill either.

Diversion of CTL

Other than an increase in dead cells owing to GMAT-driven bystander killing, GMAT can have a further complicating, and potentially negative, impact. The killing of either bystander or infected cells involves the formation of complexes with CTL. Killing of uninfected bystander cells diverts CTL from killing infected cells, which could potentially lead to an increase in viral load. If CTL are limited (which is probably the case during the initial stages of an infection) the killing of bystander cells by CTL reduces the death rate of infected cells, thus allowing these cells to produce more virus. The end result could be that despite removal of potential virus factories, the viral load increases. This would lead to more cell death by both CTL-mediated killing of bystanders, as well as virus-induced cytolysis, and presents an overall detrimental effect of GMAT.

The 'firebreak' mechanism

A possible benefit of bystander removal, which has been suggested previously [36–39], relies on a mechanism similar to a firebreak. By removing target cells around an infected cell, virions released from this infected cell must diffuse further before they encounter the next target cell. The potential impact of this mechanism depends on the spatial arrangement of target cells, as shown in Figure 3.

As the figure illustrates, the impact of the firebreak mechanism is probably negligible for situations in which the target cells are dilute and mobile; for example, CD4⁺ T cells in the blood of HIV-infected individuals. In addition, for highly mobile cells it is questionable whether contact times are long enough to establish GMAT. However, the firebreak mechanism might have a significant impact for densely packed target cells, such as epithelial cells.

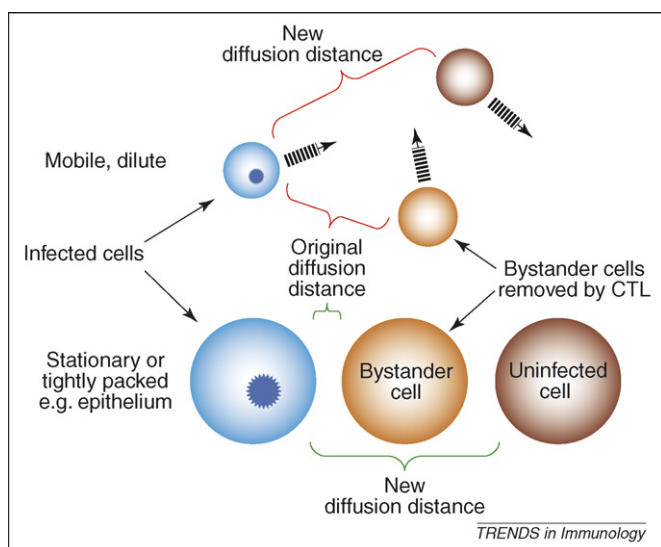


Figure 3. Schematic of the 'firebreak' mechanism. Removal of bystander cells adjacent to infected cells increases the virus diffusion distance. If target cells are mobile and the average distance between target cells is large compared to their size, removing neighboring target cells only moderately increases the diffusion distance (red brackets). However, if target cells are stationary and tightly packed (e.g. in epithelia) the new diffusion distance can be significantly larger than the original one (green brackets). Because the diffusion time is proportional to the square of the distance, in the tightly-packed case the time it takes for a virion to find a new target cell increases significantly, making it more likely for a virion to be removed before it can infect a new cell.

If the firebreak mechanism is strong enough to overcome the disadvantage of CTL diversion, then increased GMAT could lead to a reduced viral load, ultimately reducing the number of dead cells. The optimal GMAT rate will be at a level at which the firebreak mechanism is strong enough to reduce viral load significantly and thereby reduce virus-induced cell death, while at the same time keeping GMAT-induced bystander killing by CTL as low as possible.

Summary and outlook

Almost ten years ago, an article in this journal discussed the potential involvement of gap junctions in the immune response [48]. The authors concluded that although convincing evidence existed, further work was needed to clearly establish the roles of gap junctions. Since then, several studies have made progress in this area [31–35], but to our knowledge, only one *in vitro* study has suggested a possible mechanism, namely GMAT [36]. In this article, we discussed the possible impact of GMAT for different situations during a virus infection. We suggest that GMAT can potentially have an important role during activation of APC, particularly if alternative means of antigen acquisition are inefficient. The impact of GMAT between target cells is less clear. In some instances, the firebreak mechanism could result in a strong reduction in viral load and immunopathology. However, if the firebreak has little impact, GMAT could increase viral load by diverting CTL and thereby reducing killing of infected cells. We have performed some preliminary theoretical studies using mathematical models of the processes described here. For biologically plausible parameter ranges, results from the models agree with the verbal arguments. However, to obtain insights beyond the concepts discussed here, more experimental studies will be needed. Our arguments suggest that infections caused by viruses that have a latent state or that target tightly packed, stationary cells, such as epithelial or liver cells, might be a prime target for identifying increased gap junction coupling and the occurrence of GMAT.

We have focused on the potential impact of GMAT without discussing how those gap junctions are formed and how antigen transport is regulated. If GMAT is found to have an important role during certain infections, it will become important to understand the details of how GMAT is regulated. Information regarding how different types of connexins lead to differing gap junctions and what signals influence gap junction formation and GMAT will be crucial. A detailed, mechanistic understanding might open the door to manipulating immune responses during an infection, with the goal of reducing viral load and immunopathology.

We also focused here on GMAT, ignoring other potentially important roles for gap junctions during the immune response. It is possible that gap junctions have other functions in addition to GMAT. For example, they could 'alert' neighboring cells through sharing stress- or danger-signaling molecules, which might assist in the recruitment of both innate and adaptive immune components. This adds further complexity to the area, particularly if one mechanism might be beneficial whereas another – such as GMAT – might be disadvantageous.

Much remains to be clarified before we fully understand how GMAT and gap junctions influence immune responses. This area of research is relatively new, and the prospect for future studies is wide open. Because information regarding the makeup of gap junctions is available, as are molecular tools such as fluorescent dyes [6,7,23], we believe the time is right for further study in the area. We hope that the ideas presented here will stimulate further research on this potentially important mechanism of communication during immune responses.

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