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TACHYKININ-MODULATED ANTI-VIRAL RESPONSES

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1. ABSTRACT

Following viral infection, the expression of substance P and its receptor can contribute significantly to the resulting host response. For gammaherpesvirus infection of mice, the presence of this tachykinin and its receptor contributes to the protective host response. It is likely that this augmentation of the immune response is directed toward the developing T helper type 1 response and cytotoxic T lymphocyte activation. However it has also been shown that the presence of substance P and its receptor may contribute to viral diseases by facilitating viral replication or by contributing to a destructive inflammatory response. Specifically, the presence of substance P can augment replication of HIV in cultured macrophages, which is especially significant since levels of this tachykinin are elevated in patients with this viral disease. Furthermore, rodent models of paramyxovirus infection have demonstrated that the presence of neurokinin receptors and their ligands contributes to the destructive inflammatory response in airways. Taken together, these studies demonstrate a surprising role for substance P and the neurokinin-1 receptor in the host response following these viral infections.

2. INTRODUCTION

Previous reviews in this series have focused on the importance of tachykinins and their receptors during bacterial and parasitic infections. The present review will focus on our developing understanding of tachykinin modulated host responses following viral infection. Despite the fact that the studies conducted to date are limited in number, the results from such studies have been compelling. Following viral infection, the expression of both substance P and its receptor, NK-1, are upregulated. This surprising finding applies to some very diverse viral diseases, including gammaherpesvirus and paramyxovirus infections of rodents, and HIV infection. The consequences of tachykinin-mediated modulation of the host response can be advantageous or may contribute to the pathophysiology associated with that particular viral infection. Thus, the pro-inflammatory effects mediated by NK-1 have the potential to limit viral infection or augment harmful host responses following infection.

3. TACHYKININS AND THE HOST RESPONSE AGAINST MURINE GAMMAHERPESVIRUS-68

Murine gammaherpesvirus-68 (HV-68) is gamma$_2$-herpesvirus (1) which shares sequence homology and pathological similarities with Epstein barr virus (2) and human herpesvirus-8 (3, 4). As shown in Figure 1, inoculation with HV-68 (5, 6) results in a productive infection of epithelial cells, followed by latent infection of B lymphocytes (3, 7, 8), macrophages (9), and possibly dendritic cells (10) soon after inoculation. For the protective host response, early interferon alpha/beta production (11), followed by the development of a viral-specific cytotoxic T lymphocyte response (12-15), and possibly and anti-viral antibodies (16), are required. In fact, maintenance of the cytotoxic T lymphocyte response and anti-viral antibodies are thought to limit secondary disease resulting from HV-68 emergence from latency (17). The establishment of latency is associated with splenomegaly and mononucleosis that resembles that observed during Epstein barr virus infection of humans (18).

This murine gammaherpesvirus has served as a valuable model to investigate the importance of tachykinins in the anti-viral response (19, 20). Mice genetically deficient in preprotachykinin-A expression were used to demonstrate the importance of tachykinin expression following infection with HV-68 (20). Detection of infectious viral burden was approximately 100 fold higher in the lungs of preprotachykinin-A deficient mice when compared to wild type control mice. Lung pathology in the deficient mice paralleled the increased viral burden, showing increased inflammation and persistent cellular infiltrates. In addition, the level of latent virus present in the spleens of preprotachykinin-A deficient mice were significantly higher at 21 and 28 days post-infection when compared to wild type control mice. Taken together, these studies demonstrated that, in the absence of expression of the tachykinins encoded by the preprotachykinin gene, the host response controlling viral burden was significantly limited.

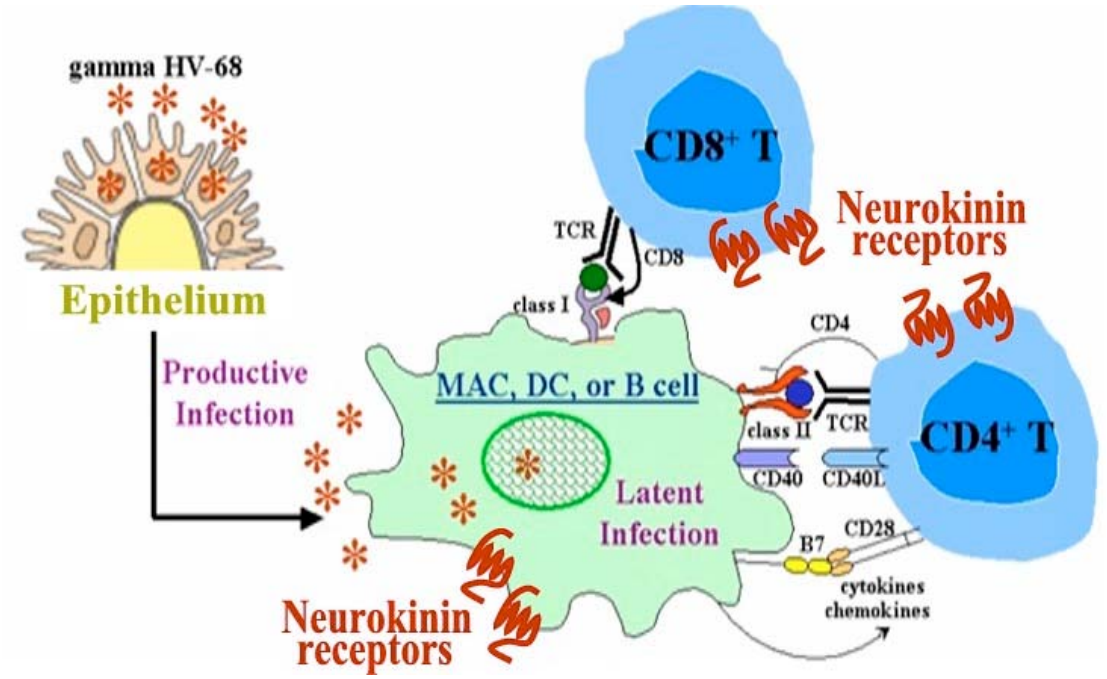

Figure 1. Possible mechanisms for neurokinin receptor-mediated immune responses against murine gammaherpesvirus-68. This diagram indicates how neurokinin receptor mediated activation of macrophages (MAC), dendritic cells (DC), B cells, CD4+ T cells, or CD8+ T cells might augment a protective immune response against murine gammaherpesvirus-68 (HV-68). Hypothetically, tachykinins could provide important signals to these cells which would augment the anti-viral response. Specifically, neurokinin receptors might signal macrophages or dendritic cells to: 1) increase secretion of interferon alpha/beta to limit spread of HV-68; 2) increase secretion of IL-12, IL-18, or particular chemokines which would favor development of a CD4+ T helper type 1 response; or 3) increase CD40, B7, and class II MHC expression to provide optimal co-stimulation for T helper cell expansion. In addition, neurokinin receptors present on CD4+ and CD8+ T lymphocytes may also augment the helper and cytotoxic functions of these cells as well. Additional studies are needed to define which of these mechanisms actually occur.

The importance of neurokinin-1 receptor (NK-1) expression during HV-68 infection has recently been demonstrated (19). Following viral infection, levels of preprotachykinin-A and NK-1 mRNA expression increased, suggesting that this ligand/receptor pair was an important component of the host response against HV-68. Such a possibility was confirmed when increased latent virus was observed in NK-1 deficient mice when compared to wild type controls. One possible mechanism that likely contributed to this increased viral burden was the fact that the viral-specific cytotoxic T lymphocyte response was diminished in NK-1 deficient mice. Together, these studies clearly demonstrated that expression of the receptor for substance P and hemokinin (i.e. NK-1) contributed significantly to a protective host response against HV-68.

While the exact mechanism for NK-1 mediated anti-HV-68 responses is not altogether clear, evidence points to a limited T helper type 1 (Th1) response as one likely pathway mediating the effect of tachykinins. NK-1 deficient mice were constructed by replacing most of exon 1 with the *lacZ* gene (21). When NK-1 deficient mice were infected with HV-68, it was possible to follow transcriptional activation of the NK-1 gene in these mice by detecting the presence of beta-galactosidase. In the spleen, marginal zone cells with a macrophage-like morphology demonstrated increased enzyme activity (19). This result could also be observed when macrophages derived from NK-1 deficient mice were exposed to HV-68. These results strongly suggested that increased NK-1 mRNA expression following viral infection occurred in macrophages. If the induced expression of NK-1 by macrophages was functional, then one would expect to observe alterations in macrophage function during infection. In fact, decreased IL-12 production was observed following viral infection of NK-1 deficient mice when compared to wild type control mice (19). Since macrophages and dendritic cells are important sources of this cytokine, it is possible that NK-1 mediated IL-12 production is an important component of the host response against HV-68.

In fact, mice deficient in IL-12p40 expression are more susceptible to HV-68 infection (22). Increased infectious virus was observed at day 9 post-infection, followed by increased viral latency in the spleens of IL-12p40 deficient mice when compared with wild type controls. It is likely that HV-68 induced IL-12 production results from the interaction of macrophages and dendritic cells with the virus since *in vitro* studies demonstrated the production of this cytokine following infection of these two cell populations (22). It has previously been demonstrated that the tachykinin, substance P, can induce IL-12 production by macrophages (23). Therefore, it is possible that tachykinin-induced IL-12 production may represent

one mechanism for induction of cytokine which would contribute to the host response against HV-68.

While the precise mechanism for tachykinin-mediated augmentation of the host response against HV-68 is developing, Figure 1 shows several possible pathways where expression of NK-1 receptors might play an important role. This diagram depicts cells and molecules which are likely to be the most important for a protective response against HV-68. Hypothetically, substance P or hemokinin which is derived from neurons or leukocytes could initially signal macrophages or dendritic cells via neurokinin receptors (i.e. NK-1) to secrete cytokines such as interferon alpha/beta and IL-12 and to upregulate expression of the co-stimulatory molecules, CD40, B7, and class II MHC. If accurate, such tachykinin-mediated responses would limit the initial spread of virus while initiating a cell mediated immune response. It is also possible that NK-1 expression by CD4+ or CD8+ T lymphocytes could contribute to the protective host response as well. While it is clear that the cytotoxic T lymphocyte response is limited following infection of NK-1 deficient mice (19), it is not clear if the diminished activity of this cell population is due to a direct or indirect action of tachykinins. Clearly many of the mechanisms presented in Figure 1 are speculative; however this diagram serves as an illustration of the studies which need to be performed to define substance P or hemokinin-mediated responses against this viral infection.

4. SUBSTANCE P MODULATION OF HIV INFECTION

During HIV infection, levels of the tachykinin, substance P, are elevated in the sera of these patients (24). In fact, infection of cultured macrophages with HIV increases the expression of this tachykinin (25). Increased substance P production during HIV infection might be part of the inflammatory host response against this viral infection, however there also appears to be some advantage for the virus as well. The same group of investigators discovered that the presence of substance P enhanced replication of HIV in cultures of human macrophages (26). This effect was specific for the substance P receptor (i.e. NK-1) since a specific antagonist inhibited infectivity of these cells by HIV (27). The possibility of using antagonists of substance P therapeutically was also suggested since treatment of cells with such antagonists could down regulate expression of substance P in macrophages that was induced by HIV infection (28). Taken together, these studies demonstrate a surprising relationship between tachykinin expression and replication of HIV in macrophages. While the expression of substance P and its receptor may contribute to the host response against this viral infection, this mechanism of inflammation seems to also favor viral replication.

5. HYPERRESPONSIVENESS OF AIRWAYS FOLLOWING PARAMYXOVIRUS INFECTION

During infection with paramyxoviruses, inflammatory responses within the airways of infected mice are mediated, in part, by tachykinins. During such viral infections, increases in substance P have been observed (29). This increase in tachykinin expression was derived from neurons since following infection, the percentage and type of neurons expressing substance P increased. The fact that substance P is a potent constrictor of smooth muscle, the increased presence of this tachykinin in airways might contribute to hyper-responsiveness during viral disease. In fact, this possible consequence of increased substance P expression seems accurate. When guinea pigs are infected with a paramyxovirus and brochoconstriction induced several days later, it was treatment with a NK-1 antagonist that could limit this constriction (30).

Studies using a model of respiratory syncytial virus infection in rats have also demonstrated the induction of NK-1 expression following lung infection (31, 32). It was suggested that this up regulation might make airways abnormally susceptible to the pro-inflammatory effects of substance P. Such a possibility was demonstrated when rats infected with respiratory syncytial virus were challenged with capsaicin to stimulate sensory neurons in the airways (33). Increased infiltration of lymphocytes and monocytes was observed, and this recruitment of leukocytes to the site of challenge could be blocked by treatment with an NK-1 antagonist. Together these studies suggest that viral infection of the upper respiratory tract can result in an increased susceptibility to inflammatory stimuli. In such scenarios, the increased expression of NK-1 and tachykinins within airways might exacerbate the pathophysiology associated with respiratory tract diseases.

6. PERSPECTIVE

The studies performed to date are significant ones since they clearly demonstrate that at least one tachykinin, substance P, and its receptor, contribute to functional responses following viral infection. In the case of murine gammaherpesvirus, the contribution made by NK-1 signaling seems to be predominately a protective one. However this may not be the case for paramyxovirus or HIV infections. Experimental models or *in vitro* studies, respectively, point toward tachykinin-mediated exacerbation of airway inflammation or stimulation of viral replication. Unfortunately, there are several significant gaps in our understanding of tachykinin-modulated anti-viral responses. Most notably is the fact that only a limited number of viral infections have been investigated to understand the possible role that neurokinin receptors might play during such microbial diseases. While the studies performed to date are compelling, the contribution made by tachykinins to the host response or to the pathophysiology of most viral infections has not been considered. This is especially true for the newly discovered tachykinin, hemokinin, which may play an even more important role in systemic viral infections due to its preferential expression within peripheral organs. Furthermore, it will be important to expand current research efforts to define the particular cellular targets for tachykinins during viral infections. This will not be an easy task since it cannot be assumed that each viral infection will effect similar leukocyte subpopulations. It is clear that

macrophages, dendritic cells, lymphocytes, mast cells, and neutrophils can express neurokinin receptors. Therefore it cannot be assumed that only one leukocyte subpopulation will be affected by the presence of tachykinins. It is likely that the induction of these neuropeptides following viral infection will have multiple effects on immune cells, as well as affecting diverse physiological functions such as vascular permeability and smooth muscle contraction, all which contribute to the complete clinical picture of viral disease.

An understanding of the mechanisms by which tachykinins and their receptors affect the host response against viruses could easily lead to therapeutic intervention. Excellent tachykinin agonists and antagonists are available for use (34), some which cross the blood brain barrier. It may be possible, for example, to limit destructive inflammatory responses that accompany some viral infections if tachykinins can be identified as significant participants in such pathologies.

7. ACKNOWLEDGEMENT

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