ABSTRACT
Objective: Our laboratory has demonstrated that the parotid gland exhibits properties of both an inductive and effector site in the mucosal immune system. While it was demonstrated that inoculation via the parotid gland produced an effective immune response and protection against a lethal dose of murine cytomegalovirus (MCMV), little is known about the molecular mechanisms of this response. Cell-adhesion molecules (CAMs) are important to the generation of immune responses as they interact with homing receptors on antigen-specific infiltrating T-cells and B-cells. There is limited data concerning expression of CAMs on normal or inoculated parotid glands. Understanding changes in CAM expression can further elucidate the mechanisms responsible for transforming the PG into a mucosal inductive site.

Methods: Mice were inoculated via a surgical incision of the PG with PBS (control) or MCMV (experimental). CAMs (PECAM, ICAM-1, MadCAM, VCAM) were analyzed from the PG and associated LNs. ICAM and PECAM mRNA were expressed earlier in the PG and later in the associated LNs. VCAM was expressed early in the PG and was maintained throughout the 21-day time course, whereas VCAM expression was expressed later in the LNs. MadCAM mRNA expression was variable (not shown). Immunohistochemistry for protein expression and real time PCR for RNA expression. Homing receptors on infiltrating T and B cells were investigated via flow cytometry using the appropriate antibodies.

Results: A statistically significant increase (p < 0.05.) in mRNA expression for PECAM, ICAM-1, and VCAM in MCMV inoculated mice versus control mice was demonstrated. However, differences in the kinetics of expression were observed between the PG and the associated LNs. ICAM and PECAM mRNA were expressed earlier in the PG and later in the associated LNs. VCAM was expressed early in the PG and was maintained throughout the 21-day time course, whereas VCAM expression was expressed later in the LNs. MadCAM mRNA expression was variable (not shown). Immunohistochemistry for protein expression qualitatively verified the mRNA results. After inoculation of the parotid gland with MCMV, significant infiltration of CD4 and CD8 T cells as well as CD19 B cells was observed. Investigations of homing receptor expression on infiltrating cells demonstrated expression of the homing receptors α1 (CD49a), α4 (CD49d), and β2 (CD29) on certain subsets of lymphocytes. PeCAM, a marker for resident tissue memory cells was also significantly induced on CD4 T cells and CD8+ T cells.

Conclusions: Our results support the hypothesis that CAM expression is modulated after immunization and differential expression of PECAM and ICAM in the PG prior to the LNs supports the role of the PG as an inductive site. In addition, infiltrating T and B cells expressed components of homing receptors that preferentially bind to either ICAM or VCAM.

Key Words: salivary glands, cytomegalovirus, cytokines, germinal centers, inductive sites.

RESULTS

Inoculation of the Parotid Gland with MCMV Induced in Lymph nodes in the parotid gland

Table 1

<table>
<thead>
<tr>
<th>CAM</th>
<th>Mouse model</th>
<th>Homing Receptor</th>
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<tbody>
<tr>
<td>PECAM-1</td>
<td>Control (vehicle)</td>
<td>CD49d</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>MCMV</td>
<td>CD49d</td>
</tr>
<tr>
<td>MadCAM</td>
<td>MCMV</td>
<td>CD49d</td>
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<tr>
<td>VCAM</td>
<td>MCMV</td>
<td>CD49d</td>
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Inoculation of the Parotid Gland with MCMV Induced Homing Receptor Expression on Infiltrating of T and B Cells

Figure 1: Histological Evaluation (H&E) of the Parotid Salivary Gland and Associated LN (SGLNs) after inoculation with MCMV. Inoculated vs. control parotid glands are represented. Values are represented as means +/- sd.

Figure 2: Cellular Infiltration of B and T cells in the parotid salivary gland LNS and the associated LNs after inoculation with MCMV. Single-cell suspensions were prepared from the parotid gland LNs and the surrounding LNs on days 14 and 28 post inoculation. A significant increase in the total number of lymphocytes from parotid gland LNs was observed on both days 14 and 28 post inoculation, whereas a significant increase in the total number of lymphocytes obtained from surrounding lymph nodes was only observed on day 28 post inoculation (left). A significant increase in total lymphocytes was also observed on day 7 post inoculation in the parotid LNs (not shown). The majority of infiltrating lymphocytes were CD4+CD44+. A significant increase in both CD8+ and CD19+ cells was also observed (right). Results are pooled from 4 mice. *P < 0.05 vs. control, Values are represented as means +/- sd.

CONCLUSIONS AND FUTURE DIRECTIONS

In this report, we demonstrated that inoculation of the parotid gland with MCMV
1. Induced CD4 and CD8 T cell and CD19 B cell infiltration in the parotid gland, the parotid gland LNs, and the associated LNs.
2. Induced the preferential expression of the homing receptors α1 (CD49a), α4 (CD49d) and β2 (CD29) on CD4, CD8, and CD19 cells, in addition, CD103 a marker for resident tissue memory cells was also significantly induced on CD4 T cells and CD8+ T cells.
3. Induced the expression of CD103, a marker for resident tissue memory cells, on CD8 T cells and CD19 B cells.
4. Induced both protein and RNA expression for ICAM, VCAM, and PECAM in the parotid gland.

These findings suggest the potential of the parotid gland to act as a mucosal inductive site with the potential to be translated as an immunization site in humans, since both homing receptor expression on infiltrating cells and CAM expression on parotid glands are coordinately induced. The long-term vision for this research will impact clinical care as that effective, DNA or other vaccine strategies, delivered through the salivary glands will be developed and provide a new and unique method of vaccination that may be especially protective against infections that start and/or progress in the mucosal membranes.