Immune Functions of Nasopharyngeal Lymphoid Tissue

Per Brandtzaeg

Laboratory for Immunohistochemistry and Immunopathology, Centre for Immune Regulation, University of Oslo, and Department of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

Abstract
This brief review will focus on nasopharynx-associated lymphoid tissue as a unique inductive immune site for B cell responses and plasma cell generation. The anatomical and immunological characteristics of Waldeyer’s lymphoid ring should make the nasal route for vaccine administration highly relevant in future clinical trials to stimulate both mucosal and systemic immunity. In this context, the potential immunological consequences of removing both the tonsils and the adenoids have to be considered.

The unpaired nasopharyngeal tonsil (also called the adenoids) and the palatine and lingual tonsils constitute most of Waldeyer’s ring. These lymphoepithelial structures appear functionally comparable to the nasopharynx-associated lymphoid tissue (NALT) in rodents, which is a lymphoid organ present on both sides of the nasopharyngeal duct dorsal to the cartilaginous soft palate [1, 2]. Waldeyer’s ring is strategically located to generate regional immunity because all of its elements are exposed to both airborne and alimentary antigens. However, although Waldeyer’s ring functions as part of mucosa-associated lymphoid tissue (MALT), it also shows similarities with lymph nodes and may, in addition, participate as effector organs of local systemic type as well as mucosal secretory type of adaptive humoral immunity.

Activation of B Cells in Germinal Centers
Primary follicles of secondary lymphoid organs such as the tonsils contain mainly recirculating naïve B cells positive for surface IgD and IgM (sIgD+sIgM+) – both co-expressed isotypes exhibiting the same specificity for antigen. Germinal centers (GCs) are hallmarks of secondary follicles generated after stimulation of naïve B cells immediately outside the follicles through cognate help from activated CD4+ T cells [3]. These helper T (T\textsubscript{H}) cells have received processed foreign antigen from dendritic cells (DCs) in the context of class II molecules of the major histocompatibility complex (MHC) – in humans also called HLA class II molecules such as HLA-DR. The stimulated B cells can then colonize the follicles and act as ‘founder cells’ for GCs.
The GCs can be divided into various more or less well-defined morphological compartments [3]. B cell stimulation in the dark zone results in exponential growth revealed by proliferation. The generated centroblasts hypermutate their Ig V-region genes and give rise to centrocytes; these cells will die by apoptosis in the light zone unless they are selected by high affinity binding to antigens on the surface of follicular DCs (FDCs). These B cells next take up such antigens and process and present them via HLA class II molecules to follicular T<sub>H</sub> (T<sub>FH</sub>) cells, which can be identified by their high level of CXC<sub>R</sub>5 expression.

Cognate interaction between T<sub>FH</sub> cells, which express the costimulatory CD40 ligand (C40L, CD154), and B cells, which express CD40, appears to be an important event in the GC reaction [3]. The same is true for the expression of bcl-2 gene products following immune activation of centrocytes to prevent their apoptosis. Indeed, if the CD40L–CD40 interaction is experimentally blocked, GCs are not formed. Importantly, this costimulatory interaction promotes switching of the Ig heavy chain constant (C<sub>H</sub>) genes of B cells from C<sub>μ</sub> (IgM) to downstream isotypes, as well as differentiation to plasmablasts and plasma cells (PCs) producing high-affinity antibody. A prerequisite for cognate interaction between B and T<sub>FH</sub> cells, is that the former express costimulatory B7 (CD80/CD86) molecules, which can bind to the CD28 receptor on T cells. Classical memory B cells (sIgD–sIgM+) with strong B7 expression are also found extrafollicularly related to the crypt epithelium where they also may exert an antigen-presenting function.

Expression of the J-chain gene occurs in a variable subset of tonsillar plasmablasts [4]. This gene encodes a 15-kDa peptide, the J (joining) chain, which is a crucial structural part of dimers and trimers of IgA (collectively called polymeric IgA, pIgA) as well as pentameric IgM. The J chain facilitates the binding of pIgs to the epithelial pIg receptor (pIgR), also called membrane secretory component (SC). The pIg-pIgR interaction is a central step in the formation and export of secretary IgA (SIgA) and secretory IgM (SIgM) antibodies [5].

**Induction of Secretory Immunity in Tonsils**

The cytokine profiles and other microenvironmental factors determining isotype differentiation and coexpression of J chain in B cells remain obscure [3]; it appears that clonal maturation in the course of several proliferative cycles results in reduced J-chain expression, thus promoting monomer production by IgA+ PCs. Whereas downstream C<sub>H</sub> gene switching in GCs of healthy tonsils gives rise to a relatively high fraction of extrafollicular IgA+J-chain+ PCs (~50% of total IgA+ PCs), most extrafollicular IgG+ PCs show little or no J-chain expression (~2%). The fact that tonsillar IgA+ PCs are mainly of the IgA1 isotype (at least >95%), along with the presence of relatively many IgD+ PCs, supports the notion that tonsillar B cell differentiation takes place mainly through classical downstream C<sub>H</sub> gene switching together with some nonclassical switching to IgD [6].

It may be envisioned that only a fraction of the pIgA-expressing plasmablasts that exit from GCs, will terminate their differentiation as extrafollicular PCs; instead, many of them may home to regional secretory effector sites for terminal differentiation to pIgA-producing PCs there (fig. 1). The adenoids possess, in addition, a local secretory immune system because patches of the crypt epithelium express pIgR/SC [2]. This is not the case in the palatine tonsils where only passive paracellular transfer of IgG and IgA takes place through the reticular crypt epithelium. Because this epithelium is of squamous type in the palatine tonsils, it is additionally protected by its production of the antimicrobial peptide calprotectin [7]. Therefore, topical surface protection differs between the two lymphoid organs.
Dissemination of Activated Tonsillar B Cells

Nasal and bronchial mucosae, as well as salivary and lacrimal glands, contain an IgA1+ and IgD+ PC distribution similar to that of the extrafollicular tonsillar compartment [3, 8]. This fact supports the notion that regional secretory tissues are seeded by GC-derived B cell blasts from Waldeyer’s ring [6, 7]. Its immune-inductive function would hence be similar to that of rodent NALT [1]. A possible minor blast contribution to the airways from bronchus-associated lymphoid tissue (BALT) remains uncertain, because organized lymphoid follicles do not regularly exist in the healthy human lung [8]. It has also been documented in many studies that nasal immunization of humans induces specific IgA antibodies in nasopharyngeal secretions, in addition to enhancing systemic immunity [3].

Attempts have been made to track directly the dissemination of B cell blasts from Waldeyer’s ring by means of molecular markers. Using a DNA marker, namely a deletion of the IgM CH gene, we showed that activated tonsillar B cells undergoing a so-called nonclassical switch to IgD+J-chain+ plasmablasts preferentially home through cervical lymph nodes to the upper airways and associated glands [9]. The extravasation of activated memory/effector B and T cells into effector tissues takes place through the local microvascular

Fig. 1. Model for T (violet) and B (green) cell induction after nasal vaccine administration, followed by generation of secretory IgA (SlgA) antibodies. (1) Delivery device for nasal vaccine administration (nasal spray, drops or OptiMist®). (2) Adjuvanted uptake of vaccine antigen through nasal mucosa. (3) Immune induction in adenoids and palatine tonsils (human NALT). (4) Antigen targeting and migration of mucosal dendritic cells (DCs). (5) Immune induction and amplification in regional (cervical) lymph nodes. (6) Compartmentalized homing/extravasation of NALT-induced T and B cells to secretory effector sites in airways, gut and uterine cervix. (7) Epithelial expression of polymeric Ig receptor (plgR) mediating external transport of dimeric IgA (plgA) to generate SlgA. As discussed in the text, NALT-derived B cells preferentially extravasate at regional effector sites and in systemic lymphoid organs, while showing only poor homing capacity for the small intestinal lamina propria.
endothelium and is controlled in a site-specific manner [8]. This process is better defined for the intestinal lamina propria than for other secretory tissues. Thus, a B-cell homing dichotomy between the gut and the upper aerodigestive tract has a molecular basis in terms of adhesion molecules and chemokines. Endothelial MAdCAM-1 is expressed throughout the gut lamina propria, where it binds gut-associated lymphoid tissue (GALT)-derived memory/effector T and B cell blasts with high surface levels of the integrin α4β7 [3].

Homing to the small intestine is, in addition, determined by the chemokine receptor CCR9 which interacts with its ligand TECK (CCL25) produced preferentially by the epithelium in that part of the gut. Conversely, homing to the colonic lamina propria is fine-tuned by CCR10 interacting with MEC (CCL25). The latter molecular pair is apparently also important for homing of tonsillar B cell blasts to the upper airway mucosa [8, 9]. These cells show poor gut-homing properties due to low levels of surface α4β7 but express, instead, CD62L (L-selectin). This adhesion molecule enables such NALT-derived cells to enter not only regional secretory tissues related to the airways but also peripheral lymph nodes and to some extent the uterine cervix mucosa [3, 9]. Integration of mucosal immunity induced in Waldeyer’s ring with systemic immunity is further enhanced by the expression of CD62L and CCR7 on tonsillar B cell blasts.

**Effect of Adenotonsillectomy on Immunity**

The above information provides strong support for the notion that Waldeyer’s ring functions as inductive NALT in humans and supply secretory effector sites of the upper aerodigestive region with activated pIgA precursor cells (fig. 1). To evaluate clinically this notion, it is important to study the effect of adenotonsillectomy on the regional SIgA levels. The pioneer report by Ogra [10] showed that combined tonsillectomy and adenoidectomy in children reduced the level of IgA antibody to poliovirus 3- to 4-fold in their nasopharyngeal secretions and delayed or abrogated their local SIgA response to subsequent live oral poliovaccine. Notably, however, this result could merely have reflected the abolished local secretory immune function due to removal of the adenoids rather than lack of regional immune-inductive capacity after the operation.

There is a need for more extensive immunological studies focusing collectively on the adenoids and palatine tonsils. Considerable redundancy of inductive lymphoid tissue in Waldeyer’s ring might mask a potentially unwanted immunological effect of adenotonsillectomy. This possibility is supported by studies that have reported reduced SIgA levels in saliva from children with pharyngitis involving recurrent tonsillitis or adenoid hyperplasia, perhaps reflecting decreased global pIgA induction in Waldeyer’s ring due to inflammation-induced downregulation of J-chain expression as seen in recurrent tonsillitis [11].

**Nasal Vaccination**

The potential advantage of nasal immunization is illustrated by the protection achieved against influenza. Many studies in experimental animals and humans have demonstrated that nasal vaccination gives rise to cross-protection against drifted influenza virus strains. With an available live attenuated influenza vaccine for intranasal administration (FluMist), good protection was achieved despite the fact that the epidemic strain was not part of the vaccine [12].

Recent reports have documented the efficacy and effectiveness of the trivalent, live attenuated nasal spray influenza vaccine (CAIV-T), both in healthy children and adults. Although nasal vaccination also efficiently induces systemic immunity, a combination of intranasal and parenteral immunization may be preferable for optimal
protection when an inactivated influenza vaccine is used [12]. Alternatively, the effect of subunit vaccines applied topically can be enhanced by incorporation into liposomes or with the addition of a nontoxic mucosal adjuvant such as Eurocine®.

References


