11
IgA and Respiratory Immunity

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11.1. General Overview of the Respiratory Immune System

Large numbers of microbes and microparticles enter the airways with every breath and the respiratory tract thus represents a major portal of entry for various viral and bacterial pathogens. In addition to mechanical defenses such as coughing, sneezing, and the action of ciliated epithelia, mucosal-associated lymphoid tissue (MALT) plays a critical role in protection of the upper and lower respiratory tracts against microbial challenge. Although the respiratory tract represents about 25% of the total 400 m^2 of mucosal tissue in the adult human, little is known about immune function in the airways. Much of our

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current understanding is actually based on information obtained from studies of the gastrointestinal system, despite the fact that many differences exist between lymphoid tissues in these two areas.

Organized nasal-associated lymphoid tissue (NALT) and bronchus-associated lymphoid tissue (BALT) are present in the upper respiratory tract and the importance of these tissues in protection against infectious disease is well accepted. However, NALT and BALT generally tend to have few germinal centers and thus resemble the isolated lymphoid tissues of the intestine rather than Peyer's patches (see Chapter 2). The lung contains little, if any, organized mucosal tissue and the dominant cellular population involved in defense in this organ appears to include alveolar macrophages rather than lymphocytes. Indeed, normal bronchoalveolar lavage fluid (BALF) consists of 90% or more macrophages. In addition, M-cells are virtually absent in the normal lung (Pabst, 1992). Lymphocytes in the lung are generally sparse and not organized, although foci of inflammation can occur during asthma and, in some cases, organized lymphoid tissues have been reported to develop in the lungs of both humans and mice during inflammation (Chvatchko et al., 1996; Delventhal et al., 1992; Tschernig and Pabst, 2000).

Antigen-specific B-cells are induced to isotype switch and undergo somatic mutation in the germinal centers of MALT inductive areas (NALT and BALT for the respiratory tract) under the influence of cytokines and microenvironmental influences (Shimoda et al., 2001; Zuercher et al., 2002). It is believed that transforming growth factor (TGF)-β drives switching to IgA, although why this is the major isotype produced by mucosal B-cells remains unknown. It is also unknown whether this switching is driven by T-cells or mucosal epithelial cells because both cell types can produce a multitude of cytokines, including TGF-β (Salvi and Holgate, 1999). In the bronchi and lungs, immunoglobulin G (IgG)-secreting B-cells are also highly represented, but it is not known whether these B-cells are activated in the MALT or draining cervical lymph nodes. After activation, B-cells home to effector mucosal tissues such as the lung under the influence of selected chemokines and adhesion molecules. Although $\alpha_4\beta_7$ and MADCAM-1 interactions appear to be critical for homing of B-cells to gut mucosal tissue, only low levels of MADCAM-1 are present on bronchial endothelial cells. Homing to the respiratory tract appears to involve $\alpha_4\beta_1$–VCAM1 interactions, the same interactions that are involved in recruiting systemic lymphocytes to sites of inflammation (Kunkel and Butcher, 2003). Nevertheless, CCR10 is upregulated on cells destined to home to the respiratory tract but is absent on systemic lymphocytes (Kunkel et al., 2003). Similarly, CCL28, the chemokine ligand for CCR10, is expressed preferentially by mucosal epithelial cells (Pan et al., 2000). Thus, CCR10–CCL28 interactions appear to direct trafficking of lymphocytes to mucosal tissues, and $\alpha_4\beta_1$–VCAM1 interactions ensure homing to the respiratory (and urogenital) tracts. This differential usage of homing receptors would explain seminal findings showing that adoptively

transferred, IgA-secreting B-cells that are obtained from the upper respiratory tract preferentially traffic to recipient airways and show only low levels of trafficking to other organs, including the gut (Husband and Gowans, 1978; McDermott and Bienenstock, 1979; Rudzik et al., 1975; Weisz-Carrington et al., 1979). However, there are likely other regulatory factors involved because IgA-secreting B-cells, but not IgM-secreting or IgG-secreting B-cells, show preferential homing to mucosal tissues.

As stated earlier, the lung has traditionally been considered to be an effector mucosal B-cell site rather than an inductive site. Jones and Ada (1986, 1987) showed that antibody-secreting cells can be expressed in the lung after influenza virus infection or immunization with inactivated virus. Furthermore, protection was correlated with the magnitude of the regional lung antibody response. That such antibody-secreting cells might actually form directly in the lung is suggested by recent studies by Randall and colleagues, who found that organized BALT-like structures are induced in the lung by influenza virus infection (Moyron-Quiroz et al., 2004). These BALT-like structures contain distinct B-cell follicles and T-cell areas and, unlike the organized lymphoid tissues in the periphery and gut (De Togni et al., 1994; Fagarasan et al., 2002), can develop in the absence of lymphotoxin-α. In fact, mice lacking lymphotoxin-α clear influenza infection more rapidly and survive higher doses of virus challenge than do their normal counterparts, suggesting that peripheral lymphoid organs actually interfere with effective mucosal immunity (Moyron-Quiroz et al., 2004). Although these BALT-like structures are only induced after infection and therefore cannot be considered constitutive, like NALT, it should be noted that even NALT is found much less frequently in humans compared to rodents. Furthermore, in the normal respiratory tract, NALT is relatively disorganized, with M-cells virtually absent. Thus, many believe that NALT also fully develops only in response to antigenic stimulation (Bienenstock, 2005). Similarly, it is known that commensal gut bacteria influence expression of intestinal IgA-producing cells and that such cells are nearly absent in germ-free animals (Hooper, 2004; Mazmanian et al., 2005; Rhee et al., 2004; Stepankova et al., 1980). This suggests that, in general, the development of mucosal lymphoid tissue requires antigenic stimulation. Finally, it should be noted that $CD28^{-/-}$ and lymphotoxin-$\alpha^{-/-}$ mice, which lack peripheral and Peyer's patch germinal centers, still can develop functional IgA antibodies in the gut (Fagarasan et l., 2001; Gardby et al., 2003; Kang et al., 2002) (see Chapter 2).

11.2. Immunodeficiency Models to Understand the Role of IgA in Respiratory Immunity

Important clues about the role of IgA in immune protection have come from patients with congenital IgA immunodeficiency, the most common human immunodeficiency disease (see Chapter 13). Although it is believed that IgA is

critical for protection of mucosal surfaces against microbial infections, IgA-deficient patients are generally not immunocompromised and usually can clear infections as effectively as normal individuals (Ballow, 2002; Burks and Steele, 1986; Burrows and Cooper, 1997). Protection in the absence of IgA is typically attributed to compensatory increases in expression of secretory IgM (SIgM) and IgG at mucosal surfaces (Brandtzaeg et al., 1987a, 1987b; Burks and Steele, 1986). Although most IgA-deficient patients are healthy, there is an increased incidence of several disorders in this population and these disorders tend to be localized to the respiratory tract. For example, the most common infections associated with IgA immune deficiency include recurrent ear infections, sinusitis, bronchitis, and pneumonia. IgA immunodeficiency also has been found to be associated with an increased incidence of allergy (Burks and Steele, 1986).

In an effort to understand further the importance of IgA in mucosal immune responses, several strains of immune-deficient mice have been developed. These include mice with genetic disruptions in the *Igh-2* gene locus encoding the α H-chain constant region and mice with disruptions in genes required for proper assembly of polymeric IgA and/or its transport across mucosal epithelia. Although human IgA deficiency appears to be caused by defects in regulatory mechanisms rather than in IgA constant region genes or genes involved in IgA transport, the murine models that have been developed do provide potentially important clues into the role of IgA in protection against microbial pathogens.

11.2.1. J-chain$^{-/-}$ Mice

The J-chain is involved in polymerization of Ig as well as the interaction of polymeric IgA and IgM with the polymeric Ig receptor (pIgR) (Hendrickson et al., 1995, 1996; Lycke et al., 1999) (see Chapter 1). Thus, one would expect decreased expression of IgA at mucosal surfaces in these mice due to altered transport across the epithelial barrier. However, it was found that the J-chain$^{-/-}$ mice created by Hendrickson et al. (1996) had levels of IgA in nasal washes and BAL (as well as breast milk and intestinal mucosal surfaces) that were similar to those in J-chain$^{+/+}$ mice. This IgA was monomeric rather than polymeric and was not bound to the secretory component. Whereas the results with these mice suggested the existence of a pIgR-independent mechanism for mucosal IgA transport, another set of J chain$^{-/-}$ mice created by Erlandsson et al. (1998) yielded contrasting results. In the latter case, the J-chain$^{-/-}$ mice had greatly reduced levels of mucosal IgA, normal numbers of IgA antibody-forming cells in the lamina propria and increased levels of serum IgA, all results that would be expected from a defect in pIgR-mediated transport (Erlandsson et al., 1998; Johansen et al., 2000). Curiously, the animals also had significantly decreased levels of serum IgM as well as IgM-producing cells, a finding that has yet to be explained. Utilizing these mice, Lycke et al. (1999) demonstrated the importance of SIgA in protection from oral cholera

toxin challenge. The potential susceptibility of these animals to respiratory infections has not been investigated to date.

11.2.2. pIgR$^{-/-}$ Mice

Polymeric IgR$^{-/-}$ mice lack the receptor necessary for transcytosis of pIg across the mucosal epithelium and, in rodents and rabbits (but not humans), hepatobiliary transport of serum pIgA (Johansen et al., 1999; Shimada et al., 1999) (see Chapter 3). Consequently, these mice completely lack SIg and concomitantly contain 100-fold greater levels of serum IgA compared to pIgR$^{+/+}$ mice. Lack of SIg in these mice does not affect development of oral tolerance following antigen feeding, nor does it influence development of cytotoxic T-cell responses after intranasal immunization (Uren et al., 2003). Furthermore, as would be predicted, systemic immunization is unaffected by the lack of pIgR, with the exception of increased serum IgA levels (Uren et al., 2005). Although these animals have reduced, but detectable, levels of IgA in the small intestine and fecal extracts, salivary IgA levels are unchanged. This latter finding indicates that pIgR function is not completely required for external IgA transport, in keeping with the results in J-chain$^{-/-}$ mice by Hendrickson et al. (1995). Nevertheless, albumin levels in saliva and IgG levels in saliva, small intestine, and feces are increased in pIgR$^{-/-}$ mice, suggesting increased mucosal permeability that presumably results from increased irritation of the mucosal epithelium by commensal organisms and ingested antigen, which, in turn, is likely caused by a lack of protective SIgA (Johansen et al., 1999) (see Chapter 10). Recent studies have implicated pIgR-mediated transport of IgA in protection against respiratory infections (see Sects, 11.4 and 11.5).

11.2.3. IgA$^{-/-}$ Mice

The development of IgA-deficient mice (IgA$^{-/-}$) has provided an extremely useful model for the further characterization of the biological activities of IgA. Disruption of the Iα exon, the α-switch region and 5′ α-heavy-chain genes cause these mice to be completely incapable of switching to and expressing IgA (Harriman et al., 1999). This deficiency was also found to cause increased levels of IgM, IgG1, and IgG2b in both the serum and gut, possibly in order to compensate for the lack of IgA. Interestingly, IgG3 and IgE levels are markedly reduced in IgA$^{-/-}$ mice, although it has not been determined if this is the result of decreased isotype switching or reduced Ig production from postswitched B-cells (Arnaboldi et al., 2005; Harriman et al., 1999). It is unlikely that disruption of the Cα gene locus would affect transcription of both Cε and $C\gamma_3$ gene regions, because whereas Cε is in close proximity to the Cα gene region, Cγ3 is far upstream. Thus, an explanation for this decrease has yet to be found. T-cells from IgA$^{-/-}$ mice also show aberrant activity, a finding that might be related to the apparent influence

of IgA on B-cell function and homeostasis, a topic that will be discussed in detail below (see Sect. 11.6).

11.3. The Role of IgA in Protection Against Viral Respiratory Infections

Intranasal infection of mice with influenza virus is a common model to examine the contributions of various immune components to respiratory tract protection. It is generally accepted that antibodies are pivotal for protection against lethal virus infection, whereas T-cells are required for recovery, although antibodies are likely involved in viral clearance as well (Huber et al., 2001; Nguyen et al., 2001a). It has been shown by Renegar and Small (1991b) that intravenous injection of pIgA anti-influenza virus hemagglutinin antibody results in transport of the antibody into nasal secretions and protection against homotypic virus challenge. IgG antibody was found not to be protective, although this might have been due to failure of the injected IgG to reach the appropriate mucosal tissues. It was further shown by these investigators that in convalescent mice, which had recovered from a sublethal infection and then were rechallenged, intranasal inoculation of anti-IgA abrogated protection induced by the priming virus, but anti-IgG or anti-IgM had no effect (Renegar and Small, 1991a). Protection in these studies was measured by quantitating levels of virus in nasal washes. This was followed by studies showing that nasal IgA depletion through chemically defined parenteral nutrition similarly led to loss of protection in immune mice, as determined by nasal virus shedding even though serum IgG antibody levels were unaffected as were numbers of IgG-secreting plasma cells in the spleens and nasal cavities of the treated mice (Renegar et al., 2001).

The advent of $IgA^{-/-}$ mice allowed the role of IgA in influenza immunity to be readdressed (Arulanandam et al., 2001; Benton et al., 2001; Harriman et al., 1999; Mbawuike et al., 1999). Naive $IgA^{-/-}$ mice are clearly not more susceptible to virus infection than wild-type animals (Arulanandam et al., 2001; Mbawuik*e* et al., 1999), but in immunized animals the situation appears to be more complicated. Initial studies by Mbawuike et al. (1999) found that after intranasal immunization with influenza subunit vaccine plus cholera toxin as adjuvant, $IgA^{+/+}$ and $IgA^{-/-}$ mice were equally protected against lethal virus challenge. Arulanandam et al. (2001) reported identical results when mice were immunized intranasally with subunit vaccine in the presence of interleukin (IL)-12 as adjuvant. An important distinction was observed, however, when immunization was performed in the absence of adjuvant. In this case, 75% of vaccinated $IgA^{+/+}$ mice survived challenge, whereas only 13% of $IgA^{-/-}$ mice survived (Arulanandam et al., 2001). Thus, it appears that the presence of adjuvant can overcome the lack of protective IgA expression. In apparent contrast to this observation is the finding that $IgA^{-/-}$ mice are fully protected from lethal virus challenge after recovery from a sublethal dose (Benton et al., 2001). This latter protection was

seen not only in relation to survival but also in viral titers in nasal washes. Curiously, $IgA^{-/-}$ mice display altered T-cell function, especially in relation to phytohemagglutinin (PHA) responsiveness (Arulanandam et al., 2001; Mbawuike et al., 1999) and development of Th1 function (Zhang *et al*., 2002b) (see Chapter 10). These mice also have defects in expression of other Ig isotypes, including IgG3 and IgE (Arnaboldi et al., 2005; Mbawuike et al., 1999). Such defects are likely related to altered B-cell antigen-presentation function in these mice, a topic that will be discussed in Sect. 11.6).

Using $pIgR^{-/-}$ mice immunized with inactivated influenza virus, it was found that protection against live virus challenge restricted to the upper respiratory tract was highly dependent on SIgA expression (Asahi et al., 2002). Protection in the nasal cavity could not be substituted by serum IgG as might be the case with infection in the lung. Similarly, Renegar et al. (2004) found that only pIgA, but not IgG, could prevent virally induced pathology in the nasal cavity, although IgG did neutralize newly replicated virus after infection had been initiated and was able to prevent pathology in the lung.

Heterosubtypic immunity to influenza virus is of immense interest for potential vaccination approaches because such immunity, if successfully induced, would allow protection against a variety of type A viruses regardless of the virus subtype used for vaccination. It is believed that subtypic immunity would best be conferred by using internal core proteins that are highly conserved among different isolates and expressed only in infected cells. As such, CD8 T-cells recognizing such internal proteins expressed by infected cells would likely be critical for subtypic protection (Nguyen et al., 1999). Indeed, after sublethal virus infection, Epstein and colleagues found that $IgA^{-/-}$ mice as well as mice lacking all Ig could control replication of heterosubtypic virus in the lungs, but depletion of CD4 or CD8 T-cells abrogated this protection (Benton et al., 2001). However, others have found that heterosubtypic immunity is dependent on B-cells. Nguyen et al. (2001b) reported that $Ig^{-/-}$ or $CD4^{-/-}$ mice primed with a sublethal dose of virus did not survive a lethal heterosubtypic or homotypic challenge, but $CD8^{-/-}$ mice did survive. Tumpey et al. (2001) found similar results using subunit vaccination followed by heterosubtypic challenge and measurement of viral lung titers. The reason for these differing conclusions regarding the role of B-cells in heterosubtypic protection is unknown but might relate to the types of B-cell-deficient mice used for the studies; Benton et al. (2001) employed specific $IgA^{-/-}$ mice and $J_H^{-/-}$ mice, whereas Nguyen et al. (1999, 2001a, 2001b) and Tumpey et al. (2001) employed *Igh-6*$^{-/-}$ (μMT) mice. The μMT strain is known to have defects not only in B-cell expression but also in dendritic antigen-presenting cell (APC) function (Moulin et al., 2000). Nevertheless, immunized $pIgR^{-/-}$ mice showed a decreased ability to clear influenza virus from the upper respiratory tract following subsequent challenge with homologous or heterologous influenza viruses (Asahi et al., 2002; Asahi-Ozaki et al., 2004). Thus, mucosal IgA does appear to play an important function in controlling heterosubtypic influenza virus infection.

The potential role of IgA in protection against other respiratory viruses such as Sendai virus (Mazanec et al., 1987) and respiratory syncytial virus

(Weltzin et al., *199*4, 1996) has been examined by passive transfer of antibody, and it has been found that both IgA and IgG are equally capable of mediating protection. Mice specifically disrupted in IgA expression or transport, however, have yet to be examined for immunity to these pathogens.

11.4. The Role of IgA in Protection Against Bacterial Respiratory Infections

The most common bacterial infections in the respiratory tract and the middle ear are caused by the encapsulated, pyogenic bacteria including *Streptococcus pneumoniae, Hemophilus influenzae*, and *Moraxella catarrhalis* (Murphy and Sethi, 1992). A mouse model of *S. pneumoniae* infection has been used extensively to determine the role of IgA in protection and it has been demonstrated that such protection can be transferred to mice with human IgA antibody (Steinitz et al., 1986). Furthermore, it has been found that human pIgA can mediate *S. pneumoniae* killing through complement receptors on phagocytes (Janoff et al., 1999).

A comparison of respiratory protection in wild-type and $IgA^{-/-}$ mice against *Shigella flexneri* was performed by Way and colleagues (1999). An attenuated form of this pathogen that is defective in oxidase expression is 100-fold less active in mediating lethal disease but can induce mucosal immunity against the fully virulent bacterial strain. However, it was found that protection was not dependent on IgA expression. A similar result was published by Murthy et al. (2004), who examined pulmonary protection against *Chlamydia trachomatis*. Infection of naïve, unimmunized mice was studied and it was found that $IgA^{+/+}$ and $IgA^{-/-}$ mice contained equivalent amounts of pulmonary bacteria 10 days after intranasal infection. In both of the above studies, protection correlated with histological inflammation in the peribronchiolar areas of the lung and, in fact, $IgA^{-/-}$ mice demonstrated more extensive inflammatory changes in response to *C. trachomatis* infection than wild-type mice. $IgA^{-/-}$ animals also expressed significantly higher levels of serum IgG antibodies, suggesting that these antibodies played an important role in protection.

In contrast to the above studies, other groups have reported a pivotal role for mucosal IgA in protection from bacterial lung infection. Lynch and colleagues (2003) found that intranasal vaccination of $IgA^{+/+}$ mice with pneumococcal polysaccharide conjugated to diphtheria toxoid induced protective immunity in adults against subsequent nasal carriage with *S. pneumoniae* type 14. The same vaccination regimen can protect neonatal mice against otitis media (Sabirov and Metzger, 2006). Such protection was not observed in $IgA^{-/-}$ mice. Dependence on mucosal IgA for protection against *S. pneumoniae* carriage was also seen using $pIgR^{-/-}$ mice (Sun et al., 2004). Lack of protection in immunized $IgA^{-/-}$ animals was directly correlated with the absence of antibody in nasal secretions. Furthermore, protection could be observed in $IgA^{-/-}$ mice

if the bacteria were first opsonized with serum antibody before intranasal challenge. It is important to note that the type 14 pneumococcal strain used in these studies does not induce inflammation in the respiratory tract nor does it cause systemic infection when instilled intranasally (Sun *et al.,* 2004). On the other hand, protection against intranasal challenge with *S. pneumoniae* type 3, a strain that does induce significant inflammation and systemic infection, does not require SIgA antibody (Sun et al., 2004). A potential caveat to the interpretation of these results is that the secretory component (the cleaved extracellular domain of pIgR) can inhibit adherence of some strains of *S. pneumoniae* to respiratory epithelium by direct binding to proteins on the bacterial cell surface, thus providing "innate" protection that is unrelated to IgA antibody specificity (see Chapters 3 and 8).

The influence of IgA in protection against *Mycobacterium bovis* BCG has also been examined using $IgA^{-/-}$ and $pIgR^{-/-}$ mice (Rodriguez et al., 2005; Tjarnlund et al., 2006). Mice were intranasally immunized with mycobacterium surface antigen PstS-1 using cholera toxin as adjuvant and intranasally challenged 2 weeks later with BCG. After immunization, no significant differences were observed between wild-type and $IgA^{-/-}$ or $pIgR^{-/-}$ mice with regard to levels of IgM or IgG antibodies in serum, saliva, or BALF (except for the absence of IgA in $IgA^{-/-}$ mice and increased serum IgA levels in $pIgR^{-/-}$ mice, as expected). Numbers of lung cells secreting tumor necrosis factor (TNF)-α and interferon (IFN)-γ were much lower in $IgA^{-/-}$ or $pIgR^{-/-}$ mice compared to wild-type controls after immunization, a finding that was distinct from the observations of Murthy et al. (2004), who quantified IFN-γ and TGF-β lung mRNA levels after *C. trachomatis* infection. Following challenge with BCG, unimmunized $IgA^{+/+}$ and $IgA^{-/-}$ mice showed essentially no differences in lung or BALF bacterial load. However, the immunized wild-type mice were able to control infection, whereas $IgA^{-/-}$ or $pIgR^{-/-}$ mice were severely compromised in this regard. Again, the $IgA^{-/-}$ and $pIgR^{-/-}$ mice produced significantly less IFN-γ and TNF-α in the lungs after infection compared to wild-type mice. Although it might be considered surprising that IgA is important for protection against an intracellular bacterium such as BCG, the results are in agreement with passive transfer studies by another group demonstrating protection against this pathogen by IgA monoclonal antibody (Williams et al., 2004). Interestingly, Reljic et al. (2006) recently reported that the protective efficacy of passively transferred IgA antibody against *M. tuberculosis* is significantly increased by coinoculation of IFN-γ.

11.5. What Can We Conclude About the Importance of IgA in Protection Against Respiratory Pathogens?

From the above discussion regarding the use of IgA-deficient animals, it is clear that different laboratories have obtained disparate results and made contrasting conclusions regarding the importance of IgA in protection of

TABLE 11.1. Protection against respiratory influenza virus infection in IgA-deficient mice.

Mouse model	Intranasal immunization	Associated inflammation?	IgA required for protection?	Comments	References
$IgA^{-/-}$	Subunit vaccine + cholera toxin	Yes	No	Cholera toxin likely induced lung inflammation	Mbawuike et al. (1999a)
$IgA^{-/-}$	Subunit vaccine alone	No	Yes		Arulanandam et al. (2001)
$IgA^{-/-}$	Subunit vaccine + IL-12	Yes	No		Arulanandam et al. (2001); unpublished observations
IgA deficient (parenteral nutrition)	Live virus to awake mice	No	Yes	Virus used for immunization likely restricted to upper respiratory tract	Renegar et al. (2001)
$J_H^{-/-}$	Live virus	Yes	No	Heterosubtypic immunity	Benton et al. (2001)
$IgA^{-/-}$	Live virus	Yes	No	Heterosubtypic immunity	Benton et al. (2001)
$pIgR^{-/-}$	Subunit vaccine + cholera toxin	Yes	Yes	Heterosubtypic immunity Measured nasal virus titers only	Asahi et al. (2002, 2004)

TABLE 11.2. Protection against respiratory bacterial infections in IgA-deficient mice.

Mouse model	Bacterial infection	Intranasal immunization	Associated inflammation?	IgA required for protection?	References
$IgA^{-/-}$	*Shigella flexneri* 2a cydC	Attenuated bacteria	Yes	No	Way et al. (1999)
$IgA^{-/-}$	*Chlamydia trachomatis*	None	Yes	No	Murthy et al. (2004)
$IgA^{-/-}$	*Streptococcus pneumoniae* type 14	Polysaccharide conjugate vaccine + IL-12	No	Yes	Lynch et al. (2003)
$pIgR^{-/-}$	*Streptococcus pneumoniae* type 14	Polysaccharide conjugate vaccine + IL-12	No	Yes	Sun et al. (2004)
$pIgR^{-/-}$	*Streptococcus pneumoniae* type 3	Polysaccharide conjugate vaccine + IL-12	Yes	No	Sun et al. (2004)
$IgA^{-/-}$	*Mycobacterium tuberculosis* BCG	Subunit vaccine + cholera toxin	?	Yes	Rodriguez et al. (2005)
$pIgR^{-/-}$	*Mycobacterium tuberculosis* BCG	Subunit vaccine + cholera toxin	?	Yes	Tjarnlund et al. (2006)

the respiratory tract from infectious agents. How can this conundrum be resolved?

The salient features of various studies analyzing protection against influenza virus in IgA-deficient mice are summarized in Table 11.1, and those with bacterial infection models are summarized in Table 11.2. Many of these models have included induction of inflammatory states, either through use of cholera toxin or live pathogen priming for immunization (sublethal doses of influenza virus) or during actual challenge (Shigella, Chlamydia, *S. pneumoniae* type 3). In general, it is the presence of inflammation that appears to determine whether IgA is necessary for any observed protection; that is, in the presence of inflammation, IgA-deficient mice are protected from infection, and in the absence of inflammation, IgA-deficient mice are not protected. This could best be explained by the fact that inflammation leads to (1) increased dendritic cell/lymphocyte activation and (2) damage to the mucosal epithelial barrier, increased blood vessel permeability, and enhanced transudation of IgG antibodies from the bloodstream. Thus, inflammation could enhance the efficacy of vaccination as well as protection upon challenge. Another variable involves the site of pathogen challenge. In the case of the lung, which has a high level of blood vessel penetration and thus IgG transudation, inflammation would tend to obscure the requirement for IgA antibody. However, in the upper respiratory tract, in which few blood vessels are present and little IgG is transudated, a requirement for IgA would become more apparent. Thus, in the studies by Asahi and colleagues (2002; Asahi-Ozaki et al., 2004), which included immunization in the presence of cholera toxin, a treatment that would be expected to induce significant inflammation, IgA was still found to be critical for protection because only virus titers in the upper respiratory tract were measured. However, in studies in which virus titers in the lung and/or survival were measured, it is likely that an amount of inflammation adequate to allow IgG transudation will be sufficient for protection. In the experiments utilizing BCG subunit immunization (Rodriguez et al., 2005; Tjarnlund et al., 2006), although inflammation was likely induced during vaccination due to the presence of cholera toxin, later bacterial challenge led to a more chronic infection in which significant numbers of bacteria were only observed 4 weeks after initial infection. This latter case contrasts with respiratory pathogens such as type 3 pneumococci, *S. flexneri*, or *C. trachomatis*, in which significant lung inflammation is typically observed within days after challenge (Murthy et al., 2004; Way et al., 1999).

From the above discussion, it appears likely that the experimental model used and the amount of inflammation induced influences the apparent need for protective IgA. The ability of IgG to substitute for IgA likely relates at least partially, to the site of infection; that is, IgA is required for protection of the upper respiratory tract, whereas both IgA and IgG can be involved in protecting the lungs. Nevertheless, the human body expends a considerable amount of energy in producing 3 g of IgA per day (see Chapter 2). It has been suggested that IgA is perfectly suited for protecting mucosal surfaces

in a noninflammatory manner brcause it doe not activate complement nor induce inflammatory reactions, and the studies in mice would tend to confirm this concept. Clearly, it would be preferable to contain infections in the lung (and other mucosal sites) before painful and potentially serious inflammation develops. However, if the endothelial and epithelial barriers are breached, IgG will transudate from the serum and serve as a backup system to prevent potential blood-borne infection. What about IgA immunodeficient humans who generally do not show any significant effects of their immunodeficiency? (See Chapter 13 for a detailed discussion of IgA deficiency in humans.) First, it must be recognized that clinical IgA immunodeficiency is defined by the presence of <50 μg/mL of serum IgA. Because we do not understand the basis for this immunodeficiency in humans but do know that α H-chains are not disrupted, it is possible that IgA-deficient individuals do contain low levels of IgA that are sufficient to protect mucosal surfaces. In addition, considering the potential for compensation by SIgM and transudated serum IgG, these individuals might have subtle defects in protection that are not recognized. In fact, it is the subset of patients with defects in both IgA and IgG expression that fare the worst clinically, and IgA-deficient mice tend to resemble this subset in showing an associated defect in isotype switching to Ig isotypes other than IgA.

11.6. IgA Expression and Mucosal B-Cell Homeostasis

Whereas it appears that IgA in the respiratory tract is important for protection against infections, its role during lung inflammation, such as that observed in asthma, has been less clear and only recently investigated in detail. Although it is widely believed that IgA can mediate protection without inducing inflammation, some reports have demonstrated a negative correlation between IgA levels and allergic sensitization (Burks and Steele, 1986; Ostergaard and Eriksen, 1979). Allergen-specific IgA can be isolated from the BALF of asthmatic patients (Nahm *et al.,* 1998) and is increased during periods of high allergen exposure (Reed et al., 1991). IgA can mediate degranulation of human eosinophils, the major cell type present in the inflammatory infiltrate of asthmatics (Abu-Ghazaleh *et al.,* 1989). Levels of CD89 (FcαR1) on eosinophils are increased in allergic patients, and IgA levels correlate with the levels of eosinophil products in BALF from these patients (Monteiro et al., 1993; Nahm and Park, 1997). Recently, the role of IgA in asthma was investigated using $IgA^{-/-}$ mice and a murine model of allergic lung inflammation (Arnaboldi et al., 2005). Induction of allergic lung inflammation in mice involves intraperitoneal sensitization with ovalbumin emulsified in alum, followed 3 weeks later by daily intranasal challenge with soluble ovalbumin. This regimen induces Th2 activation, lung eosinophil recruitment, and inflammatory changes characteristic of the early stages of human asthma, although the tissue remodeling typically seen in later stages

of human disease is not mimicked in most mouse strains. Curiously, upon immunization and challenge of $IgA^{-/-}$ mice, there were significantly reduced levels of total and IgG1 ovalbumin-specific antibodies and decreased IL-4 and IL-5 in BALF compared to $IgA^{+/+}$ controls (Arnaboldi et al., 2005). The $IgA^{-/-}$ mice also had reduced pulmonary inflammation with fewer inflammatory cells in lung tissue and BALF. Nonspecific inflammation induced by bleomycin was not affected by the lack of IgA, although the mechanism responsible for this form of inflammation is likely different from allergic lung inflammation and primarily involves infiltration of neutrophils rather than eosinophils. A comparison of $pIgR^{-/-}$ and $pIgR^{+/+}$ mice demonstrated no differences in levels of inflammation, suggesting that IgA bound to secretory component is not necessary for ovalbumin-induced lung inflammation, although a role for transudated IgA in lung secretions due to "mucosal leakiness" in $pIgR^{-/-}$ mice could not be ruled out.

Further studies in IgA-deficient mice demonstrated an important role for IgA expression in B-cell homeostasis and apparent trafficking to mucosal tissues, including the lung. First, although $pIgR^{-/-}$ mice are unable to produce SIgA due to the defect in Ig transport, they do express highly elevated levels of serum IgA. Uren et al. (2003) found that these mice had threefold greater numbers of IgA^{+} $B220^{-}$ plasmablasts in the gut lamina propria, a mucosal effector site, compared to $pIgR^{+/+}$ mice. Numbers of IgA^{+} cells in the Peyer's patches, a mucosal inductive site, were within normal range. In the converse situation, a deficiency of serum IgA was found to cause dramatically reduced B-cell numbers in mucosal effector tissues (Arnaboldi et al., 2005). Analysis of pulmonary leukocyte populations in $IgA^{+/+}$ and $IgA^{-/-}$ mice revealed significantly fewer B-cells in the lungs of $IgA^{-/-}$ mice. This difference was observed in the presence or absence of allergic lung inflammation. The decrease in B-cells was not restricted to IgA^{+} cells; there was also a complete absence of IgG-staining cells in lung sections from $IgA^{-/-}$ mice following the induction of allergic lung inflammation, a condition that generates substantial numbers of IgG^{+} cells in the lungs of $IgA^{+/+}$ mice.

What are the implications of altered B-cell homeostasis in IgA-deficient mice and why do $IgA^{-/-}$ mice show the absence of ovalbumin-induced allergic lung inflammation? The answer might lie in the known functions of B-cells other than antibody secretion, particularly antigen presentation. Indeed, it has been reported that protection against infectious diseases of the mucosa, including the lung, can be dependent on B-cells, but not Ig (Lund et al., 2003; Maaser et al., 2004). In addition, whereas transfer of whole splenocyte populations from ovalbumin-primed mice to naïve mice results in profound inflammatory responses after challenge of the recipients with allergen, removal of antigen-specific B-cells from the transferred population, either by isolating $CD4^{+}$ T-cells or by depleting $CD19^{+}$ B-cells, results in loss of responsiveness. It has also been found that both B-cells and dendritic cells are required for optimal CD4 T-cell activation, with B-cells primarily responsible for expanding the stimulated T-cell population following initial activation by

dendritic cells (Kurt-Jones *et al*., 1988; Constant, 1999; Linton *et al*., 2000; Bradley *et al*., 2002).

In the lung, antigen presentation by B cells is likely to be of paramount importance since alveolar macrophages are generally poor antigen presenting cells (Kradin et al., 1987; Leemans et al., 2005; Lipscomb et al., 1986; Lyons et al., 1986; Toews et al., 1984). $IgA^{-/-}$ mice, containing few B-cells in the lung, would thus show a defect in antigen presentation. This would explain why intranasal immunization of $IgA^{-/-}$ mice with influenza subunit vaccine plus adjuvant (to activate dendritic APC/lymphocyte function) induces protective immunity, whereas vaccination of these mice in the absence of an adjuvant results in a loss of protection (Arulanandam et al., 2001). $IgA^{-/-}$ mice show a defect in Th1 activity (Zhang et al., 2002a) as well as defective switching to IgG3 and IgE (Arnaboldi et al., 2005; Harriman et al., 1999). B-cell-deficient μMT mice as well as $IgA^{-/-}$ mice have alterations in the cytokine microenvironment compared to wild-type mice (Arnaboldi et al., 2005; Gonnella et al., 2001), including T-cell-derived cytokines. All of these findings might relate to defective B-cell expression in the lungs of $IgA^{-/-}$ mice and, consequently, loss of adequate antigen presentation and immune responsiveness. In fact, it has been found that $IgA^{-/-}$ mice lack adequate antigen presentation function for Th cell priming (Arulanandam et al., 2001).

11.7. Concluding Remarks

In this chapter, an attempt has been made to provide an overview of recent studies that have exploited murine models of IgA immunodeficiency to examine the role of IgA in respiratory immune function. In addition, an effort has been made to present a unifying concept to explain apparently conflicting results obtained from the various laboratories using these animal models. In essence, it is likely that IgA antibody provides an important first line of defense against infections of the respiratory tract. If these infections progress to a point such that significant inflammation occurs, especially in the lung, epithelial and endothelial barriers will become compromised and transudation of IgG antibody will occur and this IgG will provide a second line of defense to prevent systemic, lethal spread of the infection. Thus, the major role of respiratory IgA will be to provide protection against both morbidity and mortality, whereas IgG provides a backup mechanism to ensure survival of the host. This model predicts that IgA will be found to be necessary in experiments in which only upper respiratory tract pathogen titers are measured or protection is observed in the absence of inflammation, but IgG will appear to be sufficient for survival under highly inflammatory (i.e., lethal conditions). Simultaneously, IgA can influence B-cell expression at mucosal effector sites because overexpression of serum IgA in $pIgR^{-/-}$ mice results in increased B-cell expression in these tissues and absence of IgA causes a lack of B-cell expression. Considering the apparent importance of B-cells

for antigen presentation in the lung, their absence in $IgA^{-/-}$ mice prevents adequate pulmonary T-cell priming, which might be the reason for associated T-cell deficiencies observed in these mice. The mechanisms responsible for IgA's influence on B-cell expression and function in mucosal tissues and the relevance of the findings made in murine IgA immunodeficiency models to our understanding of human disease remain to be determined.

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