

Pointer

The application of prophylactic antibodies for rhinovirus infections

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Rhinoviruses are extremely common pathogens of the upper respiratory tract with adults experiencing on average 2–5 infections per year and children up to 12 infections. Although infections are not life threatening, except in cases of chronic lung disease where rhinoviruses are the major precipitant of acute exacerbations of disease, there is a high associated economic cost resulting from

lost productivity due to absence from work or school. Treatment of infections focuses on symptom relief with anti-pyretics/analgesics as there are no antiviral therapies available and vaccine strategies face difficulties because of the large number of viral serotypes. Here, we assess the potential for prophylactic antibody intervention for these ubiquitous human pathogens.

Therapeutic/prophylactic antibodies

Antibodies (Abs) are glycoproteins of the immunoglobulin superfamily that are produced by B-lymphocytes to foreign antigens (for review see [1]). During immune responses Abs are produced in a polyclonal fashion to antigens, although individual B-cells produce a single Ab clonotype. Importantly, in 1975 the advent of hybridoma technology permitted the isolation of B-cell clones allowing for continued production of monoclonal Abs [2]. Over the last 30 years, exploitation of their ability to specifically recognise, immobilize, destroy or clear such antigens has seen Abs transformed from diagnostic markers and laboratory tools into powerful therapeutics and prophylactics for human diseases. These Abs are usually of the immunoglobulin (Ig)G isotype due to their relatively long half-life and superior effector function as opposed to that of the IgA and IgM isotypes, which have more specialized functions. Approximately 30 monoclonal Abs are currently licensed for use to treat human diseases such as cancer, autoimmune disorders, allergy and transplant rejection [3]. Although the adaptive immune response and therefore Abs evolved to protect jawed vertebrates against infectious disease pathogens [4], to date just one monoclonal Ab (palivizumab or SynagisTM) is licensed that targets an infectious disease pathogen (respiratory syncytial virus). Nevertheless, several Abs (approximately 10 of 200) are undergoing preclinical development or are in

clinical trials for viral infectious diseases targets including HIV-1, HBV, human rhinoviruses (RV) and human cytomegalovirus [3]. RV may be particularly suited to the application of prophylactic Abs as no appropriate antiviral therapies are available despite numerous attempts [5–7], vaccine development faces challenging obstacles [8,9] and new information regarding protective antibody responses is being unearthed [10–14]. Thus, a full evaluation of the application of prophylactic Abs to these important human pathogens where there is an explicit need for appropriate therapeutic/prophylactic interventions is warranted.

Rhinoviruses and human disease

RVs are human pathogens of the respiratory tract being the most frequent cause of mild diseases of the upper respiratory tract (common cold) and the major trigger of acute exacerbations of chronic airway diseases [15,16]. In part, the success of RVs as human pathogens is due to the existence of numerous antigenically distinct serotypes and their associated heterogeneity. Serotypes were originally numbered 1 to 100 based on Ab neutralization properties with polyclonal antisera [17–19] and RVs can also be divided into three clusters (RV types A, B and C) based on sequence analysis [20]. RV type C has been recently identified due to improvements in genome sequencing and amplification

procedures and have been linked to more severe, influenza-like upper respiratory infections and severe lower respiratory illness in infants [21]. RVs can be further characterized based upon receptors used to enter host epithelial cells with approximately 90% of serotyped strains of RV (major group) using ICAM-1 as the receptor [22], whereas the minor group is known to exploit members of the low-density lipoprotein (LDL) receptor family [23]. The entry receptor for group C RVs has yet to be identified due to propagation difficulties *in vitro*.

Antibody responses to rhinoviruses and neutralizing epitopes

The icosahedral protein capsid of RVs comprises 60 copies of 4 structural proteins, the surface exposed VP1, VP2, VP3 and the buried VP4 [24]. RV-specific Abs are directed against epitopes found on the surface-exposed areas of VP1, VP2 and VP3 [25], first appearing in serum and nasal secretions 1–2 weeks following infection, peaking after 5 weeks and being maintained for over 1 year [26]. RV infections are relatively short-lived (5–7 days) and despite appearing after viral clearance, Abs are critical for protection against re-infection since defence is associated with high levels of neutralizing Ab both in serum and respiratory secretions [26]. In general, neutralizing Abs to RV are highly serotype-specific [18,19] but Abs persisting from prior encounters with other RV serotypes may cross-react with closely related RV serotypes and sometimes can cross-neutralize [9]. The importance of functional Ab responses in controlling RV infections has been recently demonstrated in patients with hypogammaglobulinaemia where, despite adequate intravenous immunoglobulin (IVIg) therapy, repeated and persistent RV infections [27] and significantly increased duration of RV shedding [28] have been documented, suggesting an important role for secretory IgA in the control of RV infections.

Numerous RV-specific murine monoclonal Abs have been generated [29–32] and rabbits and guinea pigs have been used to raise polyclonal antisera [33–36], which have aided in the identification of neutralization epitopes on the RV capsid and information on the basis of RV neutralization by Abs [25,29,37]. Structural studies showing monoclonal antibodies bound to RV indicate that effective neutralizing activity depends on Ab binding that both blocks virus–receptor interaction and stabilizes the virus capsid preventing uncoating and release of virus nucleic acid [10,38,39].

Structural [24], mutagenesis [37] and murine monoclonal Ab studies of major group RV14 and minor group RV2 [25,34] identified two neutralizing immunogenic sites (NIm-1a and NIm-1b) on VP1 that are located north of a depression on the capsid surface known as the canyon and two further neutralizing sites, NIm-2 on VP2 and NIm-3 on VP3 that are positioned

to the south of the canyon. Analogous NIm's presumably exist on numerous RV serotypes, however each NIm forms a protrusion from the virus surface and not surprisingly, these polypeptide loops are the most variable regions of the VP sequences amongst serotypes and are discontinuous epitopes, going some way to explain the high level of serotype specificity and poor cross-protective abilities of neutralizing Abs.

Recent studies have revealed that immunization of rabbits with recombinant VP1 generates antisera with broad cross-serotype neutralizing properties [13] and VP1 is the dominant target of Abs in both humans [12] and mice [11] following RV infection. VP1 is the central and most external of the RV capsid proteins, contains receptor binding sites for both the major and minor group RV [40], and has a high degree of amino acid identity within both type A and B RV serotypes [41,42]. VP1 may therefore be a good target for therapeutic or prophylactic Ab intervention for RV infections.

Prophylactic antibodies for rhinoviruses

Several potential novel strategies for neutralization of RVs by Abs are possible. These include aggregation of virions, destabilization/stabilization of virion structure, inhibition of attachment and entry of the virus into target cells by mucosal IgA or receptor blocking IgG, and binding to nascent virions to block their release from the cell surface [43]. By combining modern antibody selection techniques (phage display, B-cell immortalization and single B-cell cloning), alternative Ab scaffolds such as single chain Abs (scFv) or lamprey [44] and camelid Abs [45] with new functional assay formats it may be possible to identify antibodies that target one or more aspects of the RV life cycle. However, due to sequence heterogeneity of RV serotypes, a single pan-serotype neutralizing Ab may be extremely difficult to isolate. This could potentially be addressed by identifying several Abs with considerable breadth of cross-reactive neutralization abilities and creating an Ab cocktail. A further complication for RVs is that the aforementioned NIm's are largely serotype specific and that highly conserved capsid epitopes are not normally surface exposed and therefore unavailable for conventional Ab binding. Nevertheless, sequence conservation is found at the N terminus of the buried capsid protein VP4, which is transiently exposed at the surface in a process known as capsid breathing [5], and polyclonal Abs to this region are cross-serotype protective *in vitro* [14]. Such rare Abs may be worth further investigation as potential prophylactics, as are those Abs with the ability to interact with the conserved residues found deep in the capsid canyon – the site of ICAM-1 binding for major group RVs. However, the further potential for receptor blocking Abs of minor group RVs is complicated by the fact that the LDL receptor site is a heterogeneous

surface exposed dome formed by the interaction of VP1 subunits, whilst the capsid site and entry receptor for the RV type C group remains unknown. An approach that may circumvent these complications is to use Abs that bind to the entry receptors, thereby blocking access of RVs. Targeting the minor and major group entry receptor has been investigated [46,47] with an anti-ICAM-1 Ab approach recently described to inhibit RV infection *in vivo* [48]. One could envisage strategies where a cocktail of Abs targeting ICAM-1, LDL receptor and the RV type C receptor when identified, could have prophylactic possibilities.

Thus, the need is for novel identification and screening systems to isolate Abs with prophylactic or therapeutic potential. The recent development of a small animal model of RV infection [49] has improved investigations into potential RV therapeutic interventions and recently we have characterized the Ab responses in mice infected with RV and shown strong IgG responses to VP1 and that cross-serotype *in vitro* virus neutralization was generated [11]. Compared to studies in humans, this immunologically-naïve model allows for simpler analyses of Ab responses without interference from Abs produced from previous encounters with RVs and may simplify the identification of novel potential prophylactic monoclonal Abs to RV through the use of mice transgenic for human immunoglobulin genes [50]. Despite the challenges associated with isolation of novel human monoclonal Abs to RV, one should not overlook the potential of harnessing the power of the human Ab response, which according to Wilson and Andrews [51] is 'a rich source of highly specific, neutralising and self tolerant therapeutic reagents'. Methods for isolating human monoclonal Abs such as phage display, B-cell immortalization and single B-cell cloning are now commonly used and when coupled with high throughput screening and downstream validation approaches, may identify rare Abs with prophylactic potential for RVs.

An important consideration for potential prophylactic Abs is what the most effective isotype that provides site-specific effector function and stability *in vivo* is. Secretory IgA is the most likely candidate for protection of mucosal surfaces of the upper respiratory tract, where RV infections are most frequent but their half-life *in vivo* is relatively short. However, one should not exclude the potential importance of the IgG isotype in protection of the lungs from RV infection. In particular, infants and those with lung disease (asthma, chronic obstructive pulmonary disease and cystic fibrosis) are more susceptible to lung infections by RVs whereas healthy adults may suffer more frequently from upper respiratory tract 'colds'. Thus as the burden of disease in these lung conditions is high, it seems likely then that combination approaches of localized IgA and systemic

IgG, depending upon the recipient, may be necessary for optimal protection of RV infections by prophylactic Abs.

Conclusions and future

The major challenge facing therapeutic approaches to RV infections is the extreme heterogeneity and continued identification/evolution of new species. A prophylactic Ab approach may require combinations of serotype-specific monoclonal Abs, unconventional Abs or the application of bi-specific Abs where two different Fab arms interact in a cross-serotype manner synergistically. The immediate challenge is to attempt to identify pan-serotype neutralizing monoclonal Abs, to identify the receptor for type C RVs and to investigate alternative Ab scaffolds or formats such as IgA and scFv or the application of unconventional Abs used by camels and lampreys that allow improved efficacy. Finally, the delivery, location and stability for optimal performance of such Abs requires attention to identify what approach is best suited to combat these ubiquitous human pathogens. Systemic application of IgG may not be applicable to protect the upper respiratory tract, thus nasal administration of engineered secretory IgA in the form of a spray or mist may be of benefit. Fortunately, because RV diseases are seasonal [52] and Ab therapies have long duration pharmacokinetics, one could imagine that several applications over one winter period when exposure is predicted to be higher could afford almost complete yearly coverage. The cost of development and application of these approaches will be considerable but when taking into account the economic cost to society of RV infections in healthy subjects due to lost productivity, absenteeism from school or work and unnecessary doctor appointments, there may be significant benefits from the approaches outlined above. Benefits are likely to be even greater in those with lung diseases caused by these infections and in patients with hypogammaglobulinaemia where IVIg therapy is not sufficient.

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Disclosure statement

The authors declare no competing interests.

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