Review

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Focus: Intestinal immunity

The bilateral responsiveness between intestinal microbes and IgA

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The immune system has developed strategies to maintain a homeostatic relationship with the resident microbiota. IgA is central in holding this relationship, as the most dominant immunoglobulin isotype at the mucosal surface of the intestine. Recent studies report a role for IgA in shaping the composition of the intestinal microbiota and exploit strategies to characterise IgA-binding bacteria for their inflammatory potential. We review these findings here, and place them in context of the current understanding of the range of microorganisms that contribute to the IgA repertoire and the pathways that determine the quality of the IgA response. We examine why only certain intestinal microbes are coated with IgA, and discuss how understanding the determinants of this specific responsiveness may provide insight into diseases associated with dysbiosis.

IgA – a late bloomer

IgA has a slightly unfortunate status in immunology as a late developer. Like the third-born child in a Royal Family, it was discovered after IgG and IgM. IgA has also lived somewhat in the shadow of its sibling isotypes because it typically has a lower concentration in the serum and plays far less of a role in neutralisation of the systemic pathogens studied in classical immunology. Nevertheless, exactly as the younger scions of a Royal lineage may be esteemed for their special activities, IgA is distinguished by its prominent role in the mucosal immune system. Because most IgA is secreted across mucous membranes, its lowly serum concentrations belie the fact that it is the most-produced isotype, accounting for about three-quarters of all daily immunoglobulin secretion in mammals.

If one looks back at older review articles one finds that mucosal immunologists make much of these quantitative estimates of IgA, and of the fact that it is generally produced in different mucous membranes – one of the early pieces of evidence for a common (distinct) mucosal immune system [1]. What has been more difficult to understand is exactly how much benefit comes from investing so much energy in producing and secreting this antibody? It is well known that humans can be perfectly healthy if they are selectively IgA-deficient, and the initial reports of the targeted IgA-deficient mouse showed only a very mild phenotype, with generally normal immune responses other than compensatory alterations of IgM and IgG levels, and no difference in sensitivity to pulmonary influenza infection [2,3]. The apparent absurdity of putting so much effort into making a seemingly redundant isotype (or, in some species including humans, redundant isotypes) is highlighted by the risks involved: deposition of IgA in the renal glomeruli is the commonest cause of glomerulonephritis worldwide, leading to renal failure in about a quarter of cases [4].

One way towards resolving this paradox is to consider the role of IgA in relation to intestinal microbes. We know that IgA production is highly sensitive to the presence of intestinal microbes because mucosal IgA in nearly absent from germ-free animals and is rapidly induced upon colonisation [5,6]. The older literature also describes the ability of IgA to neutralise viruses and exotoxins, such as cholera toxin [1,7]. Looking at IgA from the perspective of microbial

Glossary

Commensal: describes bacteria that inhabit our intestine or body surface without causing harm to the host. Commensalism can however be seen as a historical term because it indicates a friendly coexistence with benefit for one party and no harm, but also no benefit, for the other. It is evident that, when considering the intestinal microbes and their host, both parties generally benefit from the relationship, and strictly-speaking this is a mutualistic existence.

Complex/diverse microbiota: the complexity or diversity of a microbiota correlates with the number of different bacterial species that are present within this microbiota. One can analyse the complexity of any microbiota by amplification and sequencing of bacteria-specific DNA with primers targeting the 16S rRNA gene variable regions. Next-generation highly-parallel sequencing methods, such as 454 or Illumina sequencing, are now used to assess which sequences are present in a sample without subcloning, and can be matched to known sequences of bacterial species.

Gnotobiotic: the term derives from the Greek words *gnostos*, 'known', and *bios*, 'life', and refers to a situation in the microorganisms within the host are defined. Germ-free mice are a special gnotobiotic case because there is known to be no microbial colonisation.

Host–microbe mutualism: the relationship between the host and its body-surface/mucosal microbes where both parties benefit from their interaction.

Pathobiont: bacteria that have a symbiotic existence within immunocompetent hosts, but may trigger pathology in particular environmental or host genomic contexts.

Reversible colonisation: this methodology allows germ-free mice to be transiently colonised with bacteria before they become germ-free again. Bacteria that allow reversible colonisation are genetically manipulated such that they are culturable *in vitro* but cannot proliferate, and therefore persist *in vivo*. This can for example be reached by making bacteria dependent on supplementation with specific amino acids which are not naturally found *in vivo*.

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Table 1. Approaches to studying the relationship between IgA and the microbiota

	Bottom up	Convergence	Top down
Definition	Studying axenic models or those with very simple microbiotas; for example, germ-free or monocolonisations	Studying defined components of complex microbiotas (e.g., IgA-bound bacteria) in a gnotobiotic system defined system	Studies of complex and natural microbiotas (e.g., human samples, SPF)
Disadvantages	(i) Limited scope (ii) May omit microbial metabolic pathways and metabolite exchanges between bacteria in complex microbiotas (iii) Mostly mouse models		(i) Imprecise definitions of microbial consortia (ii) Ambiguity in assigning effects to species (iii) Reproducibility issues (iv) Ethical issues limit human experimentation
Advantages	Molecular mechanisms and interactions between microbes or their metabolites and IgA can be defined	Defined and reproducible system with a microbiota that aims to be representative of a natural situation and is amenable to experimentation	Representation of a natural situation that models or directly shows the human situation

aggressors, production of IgA-specific proteases by some strains of *Neisseria gonorrhoeae*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* is known to be important for their pathogenicity [8]. However, such pathogens that specifically target IgA, and are targeted by specific IgA themselves, represent only the tip of the iceberg in the intestinal microbiota.

The importance of IgA directed against commensal, non-pathogenic bacteria was highlighted in elegant studies that addressed the consequences of activation-induced cytidine kinase (AID)-deficiency. Absence of AID, which results in the inability to class-switch the immunoglobulin heavy chain and to diversify the immunoglobulin repertoire through somatic hypermutation (SHM), led to an enormous expansion in the biomass of anaerobic microbes in the lower small intestine [9]. These results were refined in a second AID gene-targeted mouse strain carrying an AID^{G23S} single point-mutation where class-switch recombination was preserved, leading to normal amounts of IgA production and secretion, but SHM and the affinity maturation process were disrupted. Significantly, this strain also had microbial dysbiosis of the lower intestine [10]. These findings revealed two important characteristics of mucosal immunity. First, IgM can substitute for IgA by also being secreted across the epithelium, which explains the ability to survive without IgA, and second, SHM is required to maintain microbial homeostasis.

Nevertheless, we are left with three core questions. First, what are the organisms within the complex microbiota (see Glossary) that are targeted by IgA and which bacteria need to be targeted, and thus controlled, to maintain a healthy microbiota? Second, what happens if particular apparently benign commensals are not targeted? Third, does IgA have functional advantages over IgM other than its structural resilience in the challenging protease-rich environment at mucosal surfaces [8]? Recent studies have provided important insight into these questions, suggesting that IgA binding may be used to define components of the microbiota that have pathobiont potential in particular disease settings and, furthermore, outline approaches that enable the isolation of these bacteria. We review these findings here and place them in context of our broader understanding of the functions of IgA in intestinal immunity and the maintenance of host–microbe mutualism.

Approaches to studying the relationship between IgA and the microbiota

The diversity of the microbiota at different body surfaces leads to highly multidimensional problems in addressing the mechanisms of how we happily co-exist with our enormous load of microbial collaborators. The issue of microbiological complexity has generally been addressed from two directions (Table 1). (i) The top-down approach: where humans or experimental animals with highly diverse microbiotas have been studied. This approach has been revolutionised through the availability of highly-parallel sequencing techniques that allow correlations between the composition of complex microbial communities and immune responses. Although we are still going through an era of genomic annotation, in some cases it has been possible to pick out microbial species that are likely to be driving a phenotype that can then be studied in simpler systems. (ii) The bottom-up approach: animals are kept germ-free or associated with limited defined microbial communities [11]. In this case mechanistic questions that link specific microbes with host immunity can be addressed. The limitation of bottom-up studies is that one might obtain a limited (and possibly unrepresentative) view of the big (but fiendishly complex) picture and may miss some important metabolic exchange processes between members of the microbial community.

Of course, the object is to make these two approaches converge. For human health we need to understand why some people can live healthy lives with components of their microbiota that cause pathology while, in others, the very same microbial components cause pathology such as inflammatory bowel disease (IBD). Recent studies have provided insights into how microbe-specific IgA responses can act as an indicator to help us distinguish the effects of different microbes within complex microbiotas [12,13]. The advance presented by these studies lies in part in that they were able to address the significance of the subset of naturally IgA-targeted bacteria within the complex microbiota. By transferring purified IgA-bound microbes into germ-free animals new animal models were generated that show that these very organisms can cause conditions of inflammation under the right (host/environment/consortial composition) conditions. In other words, we are starting to converge the 'top-down' and 'bottom-up' approaches.