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T cell control of the gut IgA response against commensal bacteria

Macpherson AJ, Gatto D, Sainsbury E, *et al.* A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science* 2000; **288**:2222–6.

Abstract
The immunoglobulin A (IgA) is produced to defend mucosal surfaces from environmental organisms, but host defenses against the very heavy load of intestinal commensal microorganisms are poorly understood. The IgA against intestinal commensal bacterial antigens was analyzed; it was not simply "natural antibody" but was specifically induced and responded to antigenic changes within an established gut flora. In contrast to IgA responses against exotoxins, a significant proportion of this specific anti-commensal IgA induction was through a pathway that was independent of T cell help and of follicular lymphoid tissue organization, which may reflect an evolutionarily primitive form of specific immune defense.

Comment

In humans and most experimental mammals the gut lamina propria (LP) is the site of prodigious synthesis of the IgA isotype of immunoglobulin (Ig) and its secretion into the lumen. The major part of total Ig synthesis occurs here, leading to questions concerning the possible specific and non-specific stimuli of its production and the usefulness of this product to the host. Clearly, this typically continuous output of IgA is not constitutive as axenic (germ free (GF)) and newborn humans and other mammals display few secretory IgA plasmablasts in gut LP and minimal levels of secreted IgA in their gut lumen.[1 2] In some way, colonisation with members of the normal gut microbiota seem to initiate the development and chronic activity of certain elements of the humoral mucosal immune system.[3 4] This IgA consists of specific antibodies identifiably reactive with colonising bacteria, as well as of large quantities of IgA that cannot be shown to have been stimulated by or be reactive with particular antigens (Ags) present in food or microbes—so called "natural" IgA. In the mouse, the apparent duality of the IgA response might be explained by a difference in origin: firstly, conventional B cells (also called B2 cells) are specifically stimulated by microbial Ags and benefit from cognate interaction with Ag specific $CD4^+$ T cells. They are clonally expanded in germinal centre reactions (GCR) in Peyer's patches (PP) and mesenteric lymph nodes, and benefit from the positive selection process occurring in GCR leading to affinity maturation that results in specific IgA antibodies.[5 6] Secondly, a separate lineage of B cells, termed B1 cells, can be observed in the mouse. These cells, originally defined by expression of the surface marker CD5 and high expression of IgM, arise early in ontogeny, reside in the peritoneal and pleural cavity, and display receptor specificities mainly different from those of B2 cells.[7] B1 cells as well as B2 cells require colonisation of the gut by microbes to stimulate development into gut IgA secreting cells but they do not participate in cognate interaction with $CD4^+$ T cells or in GCR with consequent affinity maturation. B1 cells require or benefit from exogenous cytokines such as interleukin (IL)-5, IL-6, and IL-10, and are thought to be the main source of "natural" IgA.[8]

A recent paper by MacPherson and colleagues[9] put forward new ideas about the natural development of this gut IgA system. In their paper they provided evidence to show that much of this development and functioning is independent of T lymphocytes or their lymphokines, thereby minimising "bystander" contributions of T cells mediated by their lymphokines (see Wetzel[10]).

Furthermore, in this paper it was claimed that the contributions to IgA in the gut are Ag driven and specifically selected, mainly by microbial and food Ags, thus neglecting the supposed critical role of microbial polyclonal stimuli such as lipopolysaccharide,[11] in contributing to the generation of the B cell elements that function in the gut. Finally, evidence is shown that the responsible B cell subset for this "T cell independent" IgA production is well represented in neonatal and adult mice by B1 cells.

The basic phenomenon—the apparently excessive production of IgA in the gut—entreats the discovery of a rationale in terms of benefit to the host. However, calibration of the quantitative importance of the general mechanisms proposed by the two hypotheses—reasonably supposing they may both be somewhat operative—is critical in discerning: (1) whether neonates may be expected to respond to various orally encountered polyvalent microbial Ags; and (2) whether the peculiar evolution of an individual's gut microbiota (and exposure to particular food Ags) may distort the effective B cell repertoire available on encounter with any given frank or opportunistic pathogenic microbe via the gut.

In the paper by MacPherson and colleagues,[9] increased production of gut IgA, some of which reacted with Ags from commensal bacteria, was investigated using specific pathogen free (SPF) mice with rather low background levels of IgA relative to conventionally reared mice. SPF mice were then presumably "super colonised" by oral introduction of novel enteric microbes, some of which carried plasmids encoding distinct protein Ags. Generally, IgA secreting cells in gut LP increased approximately threefold and antibodies specific for particular bacterial Ags were detected by western blotting and Ag specific quantitative ELISA assays of gut washings. Similar findings have been made over the past decades using GF mice monoassociated with a particular enteric microbe or infected with an enteric virus, and evidence for GCR in PPs has supported the T cell dependence of at least part of the response.[3–5 12 13] The novel findings and interpretations by MacPherson and colleagues[9] are that SPF T cell receptor (TCR) knockout (KO) (TCR; $\beta(-/-)$, $\delta(-/-)$) mice display the same overall

Table 1 Natural IgA and specific IgA production in germ free mice monoassociated with individual bacteria[a]

	Listeria monocytogenes actA(−)[a]	*Morganella morganii*	*Segmented filamentous bacterium*	*Oochrobactrum anthropi*	*Helicobacter muridarum*
Mouse strain	GF C3H	GF C3H	GF C3H	GF BALB/c	GF BALB/c
Day after colonisation[b]	21	28	14	54	14
Total IgA (ng/ml)[c]	2200	924	2460	560	491
Specific IgA[d] (ng/ml)	320	44	33	0	4
% Specific IgA	14.6	4.8	1.3	0	0.8

[a]*L monocytogenes* actA (−) is a mutant strain of *Listeria* where the actA gene is inactive. The actA gene is important in translocation of *Listeria* across epithelial cells. *M morganii* is a gram positive commensal bacterium that can translocate into the host but has not been shown to be pathogenic.[3] Segmented filamentous bacterium is a strictly anaerobic commensal bacterium which cannot be grown outside of the host.[4] *O anthropi* is an aerobic gram negative bacterial strain which grows poorly in the intestinal tract and almost does not translocate into the host.[31] *H muridarum* is a commensal bacterium which has been described to live in the crypts of the large intestine and has no history of pathogenic properties.
[b]Time of maximal specific antibody output after colonisation.
[c]IgA production was determined in Peyer's patches (PP) and small intestinal (SI) fragment cultures by radioimmunoassay. Typical values for output of total IgA from PP and SI fragment cultures are: 3000–4000 ng/ml for CNV mice and 100–200 ng/ml for GF mice.
[d]Specific IgA production was determined by radioimmunoassay on plates that were coated with lysates derived from the involved bacteria.

responsiveness to "super colonisation" with a novel enteric microbe as do SPF immunocompetent mice, except that overall responses are only 20–30% of normal. Western blots and quantitative ELISA assays are interpreted as indicating that T cell independent gut responses encompass the same range of specificities for microbial Ags as seen normally, and that normalising for the 3–4-fold lower overall response, specific IgA responses versus particular microbial Ags are as robust in TCR KO mice as in fully immunocompetent mice. The caveat here is that assay of specific IgA antibodies in gut washings is subject to magnification due to the extensive aggregation of luminal IgA of all specificities, partly due to its association with mucins.[14] This concern affects quantitative but not qualitative interpretations. Presumably, because so many new specificities for various microbial Ags are detectable after "super colonisation", MacPherson and colleagues[9] extrapolate to the generalisation that *all* contributions to gut IgA are specific Ag selected and driven and that "natural" IgA Ab is not truly polyclonal but rather its particular set of specificities is as yet undefined. We have monoassociated GF mice with one of five diverse non-pathogenic enteric microbes and determined detectably specific and total gut IgA responses by using intestinal fragment cultures (table 1).[5 15] Even allowing for technical difficulties in identifying all specificities of a gut IgA response as reactive with particular microbial or food Ags, we find it hard to accept that 85–99% of this response, not assignable as specific and hence called "natural", is really *specifically* initiated by Ags of the colonising microbe. Rather, available evidence suggests that polyclonal stimuli, such as the lipopolysaccharide from Gram negative microbes and the less well defined ones from Gram positives, activate a polyclonal IgA response in the gut following colonisation.[4 11] Neonates, including human infants, are essentially unresponsive to microbial polysaccharide Ags until up to about 18 months in the case of humans.[16] This unresponsiveness is not predicted by the MacPherson/Zinkernagel hypothesis but is generally considered to reflect a mechanism of peripheral tolerisation operative in "virgin" T independent B cells.[17] The anergy developing in these cells can be antagonised by or prematurely prevented by encounters of B cells with lipopolysaccharide.[18] Of course, this latter non-selective stimulus could not only activate B cells polyclonally but also render them responsive to specific polyvalent microbial Ags in the gut or to ubiquitous autoantigens.[19] The importance of this mechanism remains to be evaluated.

The T cell independence of the specific gut antimicrobial response should be quantitatively evaluated. MacPherson and colleagues[9] found that the overall response in the gut was only 20–30% of normal in TCR defective mice. Generally, "T independent" responses are found to benefit from bystander $CD4^+$ T cell lymphokines, such as IL-4, IL-5, and IL-10. We find that purified B1 cells do not generate detectable gut IgA responses in SCID mice unless some Ag indifferent T cells are present. Thus the level of response of that part of the overall gut IgA response not dependent on PP GCR and cognate T cell interactions in physiologically normal mice is likely upregulated by T cells. Of course, non-lymphoid cells may provide the necessary cytokines or signals in TCR KO mice; such as eosinophils and mast cells providing IL-5 to gut B cells[20 21] or the recently described "B lymphocyte stimulator" (BLyS) molecule which is expressed by macrophages, monocytes, dendritic cells, and also T cells, that can stimulate B cells directly through the constitutively expressed "TACI/BCMA" receptor.[22 23]

In humans, especially in adults, almost all intestinal IgA plasma cells have been shown to have somatic hypermutations in their Ig genes, which is regarded as evidence for cognate T cell help and GC origin.[24 25] This would argue against a large contribution of T cell independent IgA production in humans. The presence of somatic mutations is however no definitive indicator of highly specific IgA antibodies as we and others have shown that even "natural" polyreactive IgA antibodies can originate from somatically mutated Ig genes.[26 27] Macpherson and colleagues[9] report the cloning of productive IgA genes from TCR KO and GF mice, which could solve this issue, but unfortunately the level of mutation in those genes was not mentioned.

The MacPherson group chose mouse B1 cells as a model for the gut effectors that provide the "primitive T independent intestinal mucosal IgA responses". Indeed, there is evidence that mouse B1 cells can contribute significantly to the gut IgA response by mechanisms that do not require PP GCR or cognate T cell interactions.[26 28] The MacPherson paper provides evidence that transfer of purified B1 cells to TCR KO mice can result in T cell independent IgA production in the gut. Their presentation is silent concerning the specificities of the stimulation and resulting IgA Igs. However, it is not unreasonable that mouse B1 cells, or their probable counterparts in other species, can make antibodies to gut microbial Ags. The potential specificities of their products for a defined set of microbial Ags for each cell product is well established.[26 29] The role of these particular Ags in stimulating their production is generally not known.

We now come to the most significant element of this exercise, not addressed directly in the paper by Macpherson and colleagues[9]: what is the relevance of IgA in gut secretions? A case has been made that the specific, probably T dependent, gut IgA responses to commensal bacteria act as a "shield" to block further local and systemic responses by excluding Ags (and disseminating bacteria).[30] To date, no such case has been supported for "natural", "thymus independent", or "primitive" IgA

responses, including the mouse B1 IgA responses in the gut. Although it seems likely that the prodigious "natural IgA" responses of the gut are of some value to the host—perhaps in modulating bacterial translocation of possible gut bacterial pathogens—much more study is needed.[31] In particular, the possible role of bacterial driven gut IgA in affecting the course of enteric virus infections remains enigmatic.[32]

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