How gut sampling and microbial invasiveness/noninvasiveness provides mucosal immunity with a nonmolecular pattern means to distinguish commensals from pathogens: A review

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ABSTRACT

Mucosal immunity distinguishes not only different microbial antigens but also separates those of pathogens from those of commensals. How this is done is unknown. The present view is that the pathogen/commensal determination of antigens depends upon as yet to be discovered molecular patterns. Here I review the biological feasibility that it also involves the detection of the invasive differences in their motility towards the gut wall when they are sampled by differently biased methods.

By their nature, pathogens and commensals have different motility – invasive and noninvasive – in regard to the epithelium. The immune system is in a position to detect such motility differences. This biological opportunity arises since different microbe sampling methods can "catch" different groups of microbes depending upon how their motility interacts with the epithelium. A biological method biased to sample those with invasive motility—pathogens—could be achieved by 'honey pot traps' that preferentially (but not exclusively) sample microbes that have a taxis to breaches in the epithelium. A biological method biased to sample those that are noninvasive—commensals—could be done by capturing microbes that are passively and stably residing in the biofilm "offshore" of the epithelium. Such differential sampling strategies would seem to relate to those carried out respectively by (i) M-cells (working with subepithelial dome dendritic cells), and (ii) sub- and intraepithelial dendritic cells.

The interactions of antigen presentation can be arranged so that the immune system links antigens from biased microbial sampling with pathogenic or commensal appropriate immune responses. Such immune classification could feasibly occur biologically through a winner-take-all competition between inhibiting and activating antigen presentation. Winner-takes-all types of processing classification are already known to underlie the biologically interactions between neurons that classify sensory inputs making it also plausible that they are exploited by the immune system. In pathogen identification, M-cell antigens would be activating and biofilm antigens inhibitory, and vise versa for commensal identification. This winner-take-all competition between antigen presentation would act to amplify small statistical biases in the two samples linked to invasiveness/noninvasiveness into a reliable pathogen/commensal distinction. This process would both complement, and acts as independent guarantor, upon the alternative pathogenicity/commensality recognition provided by molecular pattern recognition.

Keywords

Mucosal immunity, M-cells, Peyer's patches, pathogens, commensals, subepithelial dendritic protrusions, honey pot traps, epithelium, microbe motility, biofilm, PAMPs, immune information processing, microbe sampling, Crohn's disease, ulcerative colitis

INTRODUCTION

> It is not understood at present whether and how the host distinguishes between pathogenic and commensal bacteria (Rakoff-Nahoum et al., 2004: p. 229).
>
> Approximately 400 commensal microbial species are present in the gut lumen. So how do dendritic cells distinguish pathogens from luminal microflora? ... How DCs distinguish pathogens from luminal microflora is still unknown (Granucci and Ricciardi-Castagnoli, 2003: p.72-73).

The pathogen/commensal distinction problem

The gastrointestinal tract contains many pathogenic and commensal microbes. Much is unknown in regards to the relationship of commensals with their host, and in particular, how the host distinguishes between them and pathogens.

This ability to distinguish commensals from pathogens is important because if mucosal immunity is to ensure the host's survival, it needs to react very differently to pathogens and to commensals.

The relationship with commensals is one of tolerant coexistence with commensals residing in a biofilm "offshore" next to the gut epithelium. The importance of this commensal filled biofilm has not been appreciated partly because due to its fragility in standard histological preparation methods it has not been photographed until recently (Palestrant et al., 2004), though its existence had been earlier suggested (Sonnenburg, Angenent and Gordon, 2004).

The epithelium functions to separate the host not only from the gut commensals that reside in this biofilm but also to protect it from gut pathogens. For the adaptive immune system of the host to coexist with microbes in the biofilm, it must not react to them as pathogens, and so must have an exquisite ability to recognize individual types of microbes through their associated antigens in regard to their pathogenicity/commensality. The identification of microbes through their antigens, however, fails by itself to provide the host with the information as to whether a particular microbe is pathogenic or commensal.

Such information must, nonetheless, be acquired in some way by the immune system, since it is known that the immune system responds very differently to the antigens and substances presented from pathogens and commensals. For example, a gut commensal, *Bacteroides thetaiotaomicron*, has recently, been found to secrete a factor that inhibits epithelium inflammation (Kelly et al., 2004). The host would not risk inhibiting inflammation unless if it knew with certainty that this factor did not come from an epithelium invading pathogen. This argues that the immune system must have evolved a particularly effective but as yet unknown means of classifying microbes into pathogens and commensals.

Why PAMPs cannot work on their own

Pathogen/commensal distinction by mucosal immunity is usually attributed to pathogen-associated molecular patterns (PAMPs) that are recognized by Toll-like receptors. However, they do not provide (in spite of their name) this information—at least in a simple manner. This is because PAMPs are found not only on pathogens but also commensals (Rakoff-Nahoum et al., 2004). Indeed, the toll-like receptor recognition of commensals turns out to be essential for gut integrity and protection from injury: "the ability of TLRs to recognize commensal bacterial products is not simply an unavoidable cost of pattern recognition of infection. Rather, it has its own beneficial and crucial role in mammalian physiology" (Rakoff-Nahoum et al., 2004: p. 238).

Due to this nonspecificity of PAMPs, the presently known biological processes by which the immune system recognize pathogenicity have increasingly become appreciated as being insufficient to explain how it distinguishes pathogens and commensals: "It could be hypothesized that commensals bear an as yet unidentified PAMP that elicits an anti-inflammatory cytokine program or, conversely, lack a PAMP that is related to invasiveness and that induces inflammatory cytokine production" (Nagler-Anderson, 2001: p.63).

However, an as-yet-unknown PAMP is unlikely to provide the needed biological process due to an intrinsic limitation in the biological reliability of PAMPs: pathogens would be strongly selected to subvert any PAMPs that reliably distinguish them from commensals. Pathogens are already known to acquire a similar subversion due to natural selection in regards their ability to mimic a large number of immune cytokines and receptors. There is no reason to suppose that natural selection upon pathogens would not also be successful in mimicking any commensal distinguishing 'PAMPs'. Moreover, in regards to a molecule that would mark out pathogens,

there are diverse means to be invasive. It is unlikely that natural selection would be so constrained that it could not pick one that did not avoid making microbes molecularly unique, and so marked out to the host as pathogens.

Clinical importance

Our lack of understanding of how the host learns that an identified microbial antigen belongs to a pathogen or commensal therefore is a profound and continuing lacuna in our present knowledge about mucosal immunity. The problem, moreover, is important as this gap in our knowledge could be limiting our ability to develop therapeutic and treatment strategies for disorders such as inflammatory bowel diseases in which inappropriate pathogen-type responses happen for unknown reasons to residential commensals (Bouma and Strober, 2003). Only by fully understanding the pathogen commensal classification process can the dysfunction that causes these clinically important disorders be understood. Without this knowledge, it will be difficult to develop new effective means of treatment.

Outline of review

This review discusses and details how already established processes within mucosal immunity might distinguish pathogens from commensals without using PAMPs. Its starting point is that that while natural selection may select pathogens and commensals that lack unique molecule patterns, pathogens and commensals will by virtue of their pathogenicity and commensality have unique and different patterns of motility to the gut epithelium. This is particularly useful information if it could be obtained and incorporated into immune responses since unlike molecules (that can be mimicked), the motility that associates with pathogenicity/commensality cannot be faked, as it is directly part of what makes a microbe a pathogen or a commensal. The immune system, the review notes, does not need direct means that sense such invasive or noninvasive motility, since such motility can be indirectly recognized by the host through how such motility affects how microbes are sampled.

This review explores and discusses how mucosal immunity might arrange processes for this to create pathogen/commensal associations with antigens. This is done in terms of the processes underlying its mathematics, functionality and physiology. As such, this review is structured in terms of three levels: a computational (or mathematical) level, a functional (or mechanical) level, and a physiological (or cellular/tissue) level. This three level approach to modeling is parallel to that that has been found necessary for modeling the complex information processing that underlies the neurophysiology of vision (Marr, 1982). In this case, the mathematical level concerns how information related to the behavior of microbes can be converted into information concerning their pathogenicity and commensality. The functional level concerns how physical processes can be set up such that such information might be acquired and such classificatory information processing might be carried out. The physiological level concerns the actual cellular and tissue processes involved.

This division has several advantages. First, it enables the mathematics of the proposed system to be separated from questions of its functional realization. This is important since the general mathematical principles might apply to a wider range of processes than the particular one selected here for discussion. Second, it allows for the separation of functional questions from questions about how particular functions are in fact carried out by actual physiological processes. This is important since present knowledge about mucosal immunity is incomplete with indeed many of the suggested components only having been discovered in the last few years. As a result of this incompleteness, it is likely that any attempt to interpret the proposed functional part of the conjecture in terms of available immunophysiological processes will be at best oversimplified and omit major elements. Therefore at this level, discussion will be necessarily provisional and somewhat conjectural.

Another issue is that on theoretical grounds, one might expect mucosal immunity to engage in pathogen commensal identification in two domains: (i) that required for pathogen defense, and (ii) that required for commensal management. Dysfunction respectively of these two immune information processing system therefore would predict the existence of two types of disorder related to problems in pathogen/commensal identification. Indeed, this situation appears to be the case with the different symptomatology of the two inflammatory bowel diseases of Crohn's disease and ulcerative colitis.

This review, it should be emphasized, is not intended to suggest that molecular pattern recognition does not play a central role in the detection of pathogens. Rather that the processes explored here offer another complementary and guaranteeing means (the effectiveness of two independent means is greater than either of them alone) by which the immune system can gain information about microbial pathogenicity.

THE INFORMATION LEVEL

From a mathematical viewpoint, the immune system faces in its population of gut microbes, a joint set made out of two hidden subsets – one consisting of pathogens, and one of commensals (I ignore food antigens and 'neutral microbes'). The immune system can identify each member of this joint set as a particular individual due to their individualizing antigens. However, this does not provide information as to how to sort members of the joint set into the two hidden subsets of pathogens and commensals.

Such sorting requires an informational filter. An information filer is a process that maps part of a joint set into two further sets that link to its two hidden subsets. The informational filter does not have to be perfect provided it can (i) convert that joint microbial set into two new sample sets in which pathogen and commensal membership happens with different probabilities, and (ii) that the bias for doing this (pathogen or commensal enhancing) is known. Given such an imperfect filter, the enhanced membership of one kind of microbe in one of the two sets will be sufficient to enable it to be identified as a pathogen or a commensal. This is because if a microbe is enhanced in the set biased towards pathogens then this provides statistical information that can be used to identify it as a pathogen. To see how, consider the following sets.

The gut joint set is made of P (pathogens) and C (commensals) and the indices identify the microbe type:

{P1, P1, P2, P2, P2, P3, P3, P3, P3, C4, C4, C5, C5, C5, C6, C6, C6, C6}

The immune system sees this set with the P and C prefixes replaced by the noninformative M prefix (for microbe):

{M1, M1, M2, M2, M2, M3, M3, M3, M3, M4, M4, M5, M5, M5, M6, M6, M6, M6}

An information filter might pick out the following two sets

Sample 1: { P1, P1, P2, P2, P2, P3, P3, P3, P3, C4, C5, C5, C6, C6}
Sample 2: { P1, P2, P2, P3, P3, C4, C4, C5, C5, C5, C6, C6, C6, C6}

Which the immune system sees as

Sample 1: { M1, M1, M2, M2, M2, M3, M3, M3, M3, M4, M5, M5, M6, M6}
Sample 2: { M1, M2, M2, M3, M3, M4, M4, M5, M5, M5, M6, M6, M6, M6}

If the immune system knows that sample 1 is biased towards picking out pathogens, and sample 2 towards commensals, it is in position to identify M1, M2, and M3 as belonging to the set of pathogens, and M4, M5, and M6 as belonging to the set of commensals.

One way of extracting this information is by a winner-takes-all process that competitively amplifies input set differences into categorical distinctions about their members (Indiveri, 1997). Processes converting bias information into categorical information by winner-takes-all process have already been modeled for physiological systems in the form of interactions occurring in neural networks (Hahnloser, Sarpeshkar, Mahowald, Douglas and Seung, 2000; Indiveri, 1997).

A host therefore will possess the information to identify pathogens and commensals, if it can possess, (i) an informational filter that creates two sample subsets, and (ii) a means to convert the information in these sample subsets through some kind of implementation of a winner-takes-all process.

In the following sections, the discussion upon the functional and physiological levels of how this is carried out is subdivided into: (i) sampling (which provides the raw data), and (ii) winner-take-all information processing (which extracts their hidden information).

THE FUNCTIONAL LEVEL: SAMPLING

The mucosal immune system can create an information filter by how it samples gut microbes. Microbes and their antigens are known to be sampled by the mucosa in several ways (discussed below). However, in present immunological theory about sampling, these are considered to be part of one general type of process. This is reflected in the lack of discussion of how different kinds of sampling might function as informational filters. Without such discussion, it is difficult to appreciate that sampling cannot only catch microbes but also obtain different kinds of information about their pathogenicity/commensality. If this is not appreciated, it is difficult to understand how mucosal immunity might be organized to biologically process this information.

Microbial behavior and pathogenicity/commensality

Commensals and pathogens benefit and harm respectively the host. This differences is closely linked to their motility towards the host's epithelium. For a pathogen to exploit (and so harm) the host, it must in some way attach, invade or penetrate the epithelium. Commensals, in contrast, to provide the host with benefit need only passively reside adjacent to the epithelium in its associated biofilm. Thus, one way the immune system might gain information to classify a particular microbe as pathogenic or commensal is in terms of the invasiveness of their motility to the epithelium.

The mucosal immunity system cannot directly observe the microscopic behavior of microbes since it lacks the appropriate sense organs to detect their movement. Moreover, the lumen is congested with microbes making the identification of any particular one and its motility intrinsically difficult.

However, a microbe's motility can be indirectly known at least in regards its motility to the epithelium since this motility effects how they might be caught.

For a microbe to exploit the host, (and so be pathogenic), it needs to actively cross the layers of commensals, mucus, biofilm, and glycocalyx that protect the host's epithelium so it is in a position to either disrupt the epithelium, or gain access to the host's interior. If a microbe cannot physically move through the mucus, or find the epithelium where its protective layers are disrupted, then it is unlikely to be in a position to harm the host, and so effect it as a pathogen. Therefore for a microbe to be a pathogen, it must in someway engage in actively locating unprotected epithelium, or have a means to actively swim through its protective layers.

In contrast, commensals have no need to physically interact with epithelium cells as they can reside and replicate in the epithelium associated biofilm (for arguments that commensals reside offshore of the epithelium in the mucus, see Sonnenburg, Angenent and Gordon, 2004; evidence for biofilm, Palestrant, Holzknecht, Collins, Parker, Miller and Bollinger, 2004, also see endnote 1 on segmented filamentous bacteria).

Microbe motility and the opportunity to distinguish pathogens and commensals

Pathogens and commensals therefore differ markedly in regard to one aspect of their motility: how close they seek to get to the epithelium, and in particular, how they behave to weaknesses in the protection over the epithelia. Pathogens will seek these out, while commensals will be indifferent to such weaknesses.

These differences in motility can be identified by sampling microbes (i) where there is no or little protection, and (ii) where it is strong. A sample captured from where protection was weak could be expected to catch more pathogens than where it was strong, since pathogens by their nature will seek out weak areas for invasion. Thus, the information for distinguishing commensality and pathogenicity of microbes can be obtained by separating microbial sampling into two methods: one that differentially picks up microbes depending upon whether they actively seek to venture through the mucus and across the glycocalyx, and another one that samples only those that seek to reside passively offshore in biofilm. Such a combination of samplings that are contrastingly biased to different microbial behavior would provide the immune system with a statistical differences with which to classify sampled microbes in regard to their pathogenicity/commensality.

Two samplings methods are proposed here:

(1) "Honey pot traps" that select for microbes that seek out the epithelium; and
(2) Random capture of microbes found in the epithelium associated biofilm that forms in the unstirred layers (glycocalyx and mucus) adjacent to the epithelium.

Honey pot traps

The term 'honey pot traps' is a computer term used particularly in the context of spam detection and network security. Honey pot traps in such a context are deliberated weakened parts of a system that are monitored to provide a warning of an attack. In network security, easily entered but nonfunctioning "bait" parts of the computer system are set up and monitored. These computer system honey pot traps thus allows attacks by hackers to be detected before critical and important areas of the system are compromised. This provides system operators with a warning of system security failings without actually opening up the system to risk. The makers of spam filters similarly set up real but unused email accounts that trick spam spiders into collecting their addresses so they are sent unsolicited emails. Email sent to such honey pot traps can then be used to construct spam filters with no risk of misclassification.

Functionally, several things would be required of any gut immunity honey pot trap.

- It must provide an early warning by offering what appear to pathogens to be 'epithelium' cells that they will reach prior to them mounting an attack on the real epithelium. Since the real epithelium is covered by several protective layers such as brush border, glycocalyx, biofilm, mucus (unstirred and stirred) and secreted antibacterials, these must be weakened or absent above such honey pot trap cells. Due to such "weakening", such 'unprotected' cells would be preferentially "attacked" before the protected epithelium.

- Since mucus and protective layers can be abraded off by food particles, the epithelium will often be exposed to lumen pathogens. The protective layers of mucus above the honey pot trap thus should be arranged so that they are more readily abraded than that over the epithelium to allow lumen based pathogens to be detected ahead of their attack on actually exposed epithelium.

- While honey pot trap cells might express macromolecules that attract mobile microbes, they must not carry specific surface markers that might distinguish them from epithelial cells. If they did, this might warn pathogens that were contacting non-epithelium sampling cells. Selection during selection, it is reasonable to suppose, would strongly advantage any pathogen able to detect a marker present on such a sampling cell, and then use its mobility to avoid capture. This is important not only for the microbe's own individual survival but in aiding the survival of its microbial type by preventing the host gaining an early warning that they are present in the lumen and about to mount an attack.

- Mucus is highly complex and contains at least two layers (unstirred and stirred) (Matsuo, Ota, Akamatsu, Sugiyama and Katsuyama, 1997). Further, the inner unstirred mucus contains biofilm layers. It is likely that pathogens are able to detect biochemical and hydration graduations to guide them to the epithelium. The honey pot trap should therefore secrete substances to attract pathogens such as carbohydrate fragments associated with the mucus that are used by pathogens in chemotaxis to locate or orientate towards the epithelium (O'Toole, Lundberg, Fredriksson, Jansson, Nilsson and Wolf-Watz, 1999).

- It must actively seize microbes through engulfment that will capture microbes that would otherwise use their mobility to escape.

Microbes sampled at a honey pot trap will be mainly nonpathogenic microbes brought into contact with it accidentally by the peristaltic stirring of the mucus. Further, such microbes will vastly out number pathogenic ones with the result that even if only a very small percentage of them end up at honey pot traps, they will in absolute terms be the main sampled microbes. The capture by a honey pot trap of a microbe therefore cannot by itself provide the information to identify it as pathogenic. Moreover, this inability to detect pathogens due to the large numbers of bystander nonpathogenic microbes will reduce its sensitivity to pathogens in the critical period when are small in number and so, if detected by the host, most easily cleared. Thus, a honey pot trap must be complemented by another "control" method of sampling with a different bias in its microbial capture.

Biofilm sampling

Several protective layers emanate outwards from the epithelium in which microbes can potentially reside or contact.

- First, epithelial cells are covered with microvilli that form a bush layer that is difficult for microbial attachment but across which small molecules can freely diffuse.

- Second, coating these is the glycocalyx which consists of a 400-500 nm layer of membrane-anchored glycoproteins, glycolipids and mucin-like molecules.

- Third, the lower or unstirred mucus layer produced by goblet cells and epithelium cells.

- Fourth, the upper or stirred mucus layer that is horizontally stratified due to lubricating side movement with the passage of the lumen.

Commensal microbes reside mostly offshore from the apical surface of the epithelium (Sonnenburg, Angenent and Gordon, 2004) in biofilm (Palestrant, Holzknecht, Collins, Parker, Miller, and Bollinger, 2004) in the

unstirred mucus layer though others more transiently will be found in the stirred layers. In the unstirred mucus, the microbes adjacent to the epithelium in biofilm can be directly sampled by cells either located in or below the epithelium. This sample will be a biased not to contain pathogenic microbes as they normally will be comparatively rare in the mucus containing commensal biofilm.

Moreover, commensals unlike pathogens will not be so mobile, particularly when embedded in biofilm. As a result, they can be caught without engulfment. This provides a further filter that will differentially sample commensals in preference to pathogens: cells that sample microbes directly using extensions cannot actively engulf microbes, and so will be biased to catch immobile ones (mobile microbes will be able to move away or evade such extensions).

One limitation on the pathogen/commensal distinction processes as presented here is the omission of a role for inflammatory cytokines. This is done so that the information process described at the mathematical and functional levels can be shown to exist in physically plausible processes. Further, microbes will also be sampled after open wounds let in pathogens and commensals into the lamina propria below the epithelium. The role of inflammatory signals and such wound sampling need to be incorporated in a more refined and developed model.

PHYSIOLOGICAL LEVEL: SAMPLING

M-CELLS ARE MUCOSAL IMMUNE SYSTEM HONEY POT TRAPS

M-cells are specialist antigen gathering cells of the follicle–associated epithelium (Kato and Owen, 1999; Kraehenbuhl and Neutra, 2000). Below them are T and B cells, macrophages and dendritic cells of the mucosa-associated lymphoid tissues (MALT). These lymphoid follicles are grouped in dome like areas known as Peyer's patches. The antigen gathering of M-cells works closely with the transfer of such antigens to professional antigen presenting cells. A distinctive feature of M-cells is a basolateral intra-epithelial 'pocket' that shortens the distance (which can be as small as 1-2 µm) traveled by transcytotic vesicles transferring microbes and their antigens from M-cells to "docking" lymphocytes and dendritic cells.

This review assigns a new function to M-cells. At present, they are considered to be specialist "port holes" in the epithelium that catch microbes and other antigens for dendritic cells below them to sample. In this traditional view, such specialist portals are needed because epithelium cells provide an effective barrier that stops the immune system otherwise being able to sample the gut. However, the immune system, it is now known, can sample the gut through subepithelial and intraepithelial dendritic cells (see below) which suggests a more complex function. Moreover, on the apical lumen side of M-cells is a specialist mucus environment which acts to enhances their ability to catch epithelium seeking pathogens. Peyer's patches in which M-cells reside do not produce protective factors, mucus (though this flows from elsewhere) nor IgA, and so they are likely not to be covered by commensal biofilm. This associated weakened mucus environment is one that will be easily penetrated by mobile microbes seeking to invade the epithelium. I suggest, therefore, that M-cells act not only as immune portals to obtain gut contents have also evolved a function as honey pot traps.

Honey pot traits of M-cells

M-cells have several characteristics which suggest that their sampling functioning is enhanced for the purpose of making them effective honey-pot traps.

Resemble vulnerable epithelium targeted by pathogens

M-cells, as noted, unlike epithelial cells are relatively unprotected. Epithelial cells are covered by a brush border, glycocalyx, unstirred and stirred mucus layers, and biofilm. The unstirred mucus above the epithelium shows evidence of deriving from goblet and epithelium cells (Matsuo, Ota, Akamatsu, Sugiyama and Katsuyama, 1997). Goblet cells are absent in Peyer's patches (Owen and Jones, 1974). As a result, the mucus covering the Peyer's patches will have to be of the stirred kind derived from elsewhere, and so likely to lack the protection offered by the commensal microbial biofilm which exists in the more inner unstirred mucus layers. This is not just physical protection: microbes produce antimicrobial substances to aid their survival against competing microbes (Padilla, Brevis, Lobos, Hubert and Zamorano, 2001). It is likely that these antimicrobial substances are found in epithelium associated biofilms providing a protection in addition to its offering of a physical barrier. M-cells do not have a brush border and either a weakened or absent glycocalyx coating (Neutra, Mantis, Frey and Giannasca, 1999; Lamm, 1997). While M-cells readily take up secretory IgA, none is secreted in the Peyer's patches, and indeed by the epithelium cells flanking them (Pappo and Owen, 1988). IgA contributes to biofilm formation (Sonnenburg, Angenent and Gordon, 2004) so providing another reason for supposing the absence of biofilm above M-cells.

Placed in a position to get pathogen activity

M-cells associate together as part of a shaped structure, the dome region of follicle–associated epithelium. This shape could further reduce the protection over M-cells by making the mucus (and its associated biofilm) covering them preferentially abraded compared to that over the epithelium cells. Moreover, it is possible that the shape of the dome area induces local changes to the mucus flow that might act to direct active mobile microbes to M-cells. Mucus can be produced in different degrees of hydration or other respects such that peristaltic stirring will structure it locally so that mobile microbes are guided preferentially (in combination with different gradients of such bioactive substances as defensins) to M-cells rather than to epithelium cells.

Can catch mobile microbes

The apical surface of M-cells is active containing "variable microfolds interspersed with large plasma membrane subdomains that are exposed to the lumen [that] .. have been shown to mediate endocytosis of ligand-coated particles, adherent macromolecules .. [and] .. fluid-phase pinocytosis, actin-dependent phagocytosis, and macropinocytotic engulfment involving disruption of the apical cytoskeletal organization (Kraehenbuhl and Neutra, 2000: p. 308-309). The active nature of the M-cell's apical surface allows it to pick up in addition to passive entities, mobile ones that might seek to evade capture. M-cells could indeed be responsive to movement as live *Vibrio cholerae* are quickly taken up but not *V. cholerae* that have been killed (Owen, Pierce, Apple, Cray, 1986). It should be noted that this could also be due to dead *V. cholerae* shedding some as yet undiscovered adhesins (Neutra, Mantis, Frey and Giannasca, 1999: p.174). Such active methods would coexist with ones that selectively pick up immobilized microbes. M-cells express an IgA-specific receptor on their apical surfaces and selectively take in IgA but not IgG or IgM coated antigens (Mantis, Cheung, Chintalacharuvu, Rey, Corthesy and Neutra, 2002).

Molecular stealthiness

(1) In spite of many efforts, no specific surface marker has been found for M-cells (Wong, Herriot and Rae, 2003; Clark and Hirst, 2002; Neutra, Mantis, Frey and Giannasca, 1999). They are instead histochemically revealed with substances such as UEA-1 that also attach to mucus. This usually does not interfere with their histological visualization as mucus is not normally left attached to the epithelium unless special and complex precautions are taken (such as the flash freezing of tissue samples in nitrogen). The hunt for M-cell specific makers has been a major research concern since without them they cannot be isolated by cell fractionation and this complicates their study by immunohistochemical methods. One reason for them lacking such a marker, I suggest, is that if they did, natural selection would select microbes that could detect it and so engage evasive mobility when they made contact with M-cells. To act as honey pot traps, M-cells must have "molecular stealth" least they give their presence away to microbes.

(2) M-cells display proteins with carbohydrate side chains (including lectin UEA-1 binding sites) that partially characterize mucin (Lelouard, Reggio, Mangeat, Neutra and Montcourrier, 1999; Lelouard, Reggio, Roy, Sahuquet, Mangeat and Montcourrier, 2001). Their presence is usually explained in terms of their binding microbial adhesions (Giannasca, Giannasca, Falk, Gordon and Neutra, 1994). However, pathogens are chemotaxic for differential mucin concentrations suggesting that these carbohydrates may serve as attractors (O'Toole, Lundberg, Fredriksson, Jansson, Nilsson and Wolf-Watz, 1999). Further, M-cells display enhanced expression of certain markers (non-unique) such as junction-associated proteins (Clark and Hirst, 2002). This might be another factor with which to either to attract pathogenic microbes or mislead them into reacting to M-cells as if they were epithelial cells.

(3) Though it has gone without comment, it is theoretically significant that M-cells are not themselves antigen presenting cells. Why should the major cell for capturing microbes and their antigens not have this further function and instead be specialized (as demonstrated in their basolateral docking pockets) for quickly passing bacteria and their antigens on to professional antigen presenting cells? The constraints of their role as honey pot traps could provide an explanation: a honey pot trap cannot express markers (as they would, if they were made up of antigen presenting cells) that would warm a pathogen that it might be caught for antigen analysis. However, antigen presenting cells seeking to capture nonpathogenic microbes need not be disadvantaged (commensals do not suffer if the host learns of their presence in the biofilm) and so can be involved in sampling them. M-cells would seem a specialized adaptation to get around this problem for the sampling of pathogens: the two functions of antigen display and antigen capture have to be separated if there is to be stealthy pathogen capture.

A possible objection against M-cells functioning as honey pot traps

It might be objected that M-cells are notable as the entry point for several pathogens such as *Salmonella typhimurium*, *Listeria monocytogenes*, and *Shigella flexneri*. This penetration requires the virulence factor of invasion proteins. If M-cells were honey pot traps, this would suggest they also do so at the cost of providing an entry port for pathogens that can subvert this function. This, however, does not conflict with the idea that M-cells are in fact honey pot traps. Rather it suggests that the openness used to entrap pathogens is done at the cost of enabling some pathogens to gain easier access across the epithelium. Such a cost would only be evolutionary maladaptive if it was greater than the benefits of another function—such as the one proposed here—of creating an early warning system for pathogens. Moreover, though it does not provide a complete defense, evolution has attempted to minimize pathogens gaining access though M-cells: large numbers of macrophages exist below M-cells that can eliminate rouge pathogens.

SUB- AND INTRAEPITHELIAL DENDRITIC CELL SAMPLING OF BIOFILM

Dendritic cells exist that could sample microbes and microbial antigens in the biofilm area adjacent to the epithelium. These dendritic cells seem to be of two kinds. First, there are subepithelial dendritic cells present in the lamina propria that send up protrusions that pass through the tight gaps between epithelial cells, and even venture outside of the epithelium while remaining still in contact with it (Rescigno et al., 2001; Iwasaki and Kelsall, 2001: p. 4889). Second, intraepithelial dendritic cells exist basolateral to epithelial cells (Maric, Holt, Perdue and Bienenstock, 1996). Little is known about such intraepithelial dendritic cells. It is not known whether and how such intraepithelial cells sample the zone adjacent to the epithelium, nor whether they are of the same dendritic subtype as the intraepithelial dendritic cells that send up protrusions between the tight gaps of epithelial cells. Further, it is not known whether they form networks as found for the intraepithelial dendritic cells located in the lung (Schon-Hegrad, Oliver, McMenamin and Holt,1991), and nasal mucosa (Jahnsen, Gran, Haye and Brandtzaeg, 2004).

The extent to which such protrusions from dendritic cells reach out beyond the apical surface of the epithelium is also unknown. (Though it is interesting to note that in another context – communicating antigens to T cells – that dendritic cells send out protrusions as long as 50 µm, Boes et al., 2002.)

Sub- and intraepithelial dendritic cell sampling as commensal biased

Sub- and intraepithelial dendritic cell sampling—even if confined to the glycocalyx and inner biofilm area immediate to the apical epithelial surface—would select a markedly different population to that sampled by M-cells located in Peyer's patches. This sampled population would be characterized by microbes that resided in adjacent biofilms, or that by random process had been moved close to the epithelium. As noted above, pathogens are mobile and would presumably avoid capture by dendritic protrusions. Further, such protrusions would contain markers identifying them that they were not epithelial cells and so warn off potential pathogens. Thus, this form of sampling would be biased to pick up commensals resident in the mucus.

Limits upon our knowledge about sub- and intraepithelial dendritic cell sampling

Our present knowledge of sub- and intra epithelium dendrites is limited. There is only limited citation of John Bienenstock's group work upon intraepithelial dendritic cells (Maric, Holt, Perdue and Bienenstock, 1996). Though widely discussed (including several commentaries, Collins, 2001; Gewirtz and Madara, 2001), only two empirical papers have been published upon direct dendritic cell sampling (Rescigno et al., 2001; Rescigno, Rotta, Valzasina and Ricciardi-Castagnoli, 2001).

Gut sampling therefore could easily be more diverse than that suggested above. Recently M-cells with associated lymphoid follicles have been detected that are independent of Peyer's patches (Jang et al., 2004) that exist spread out along rows (Hamada et al., 2002). It is not clear what they might doing but they could be offering another kind of sampling to aid the status identification of microbes. M-cells, moreover, are not homogeneous and might specialize in the sampling of different subsets of microbes (Neutra, Mantis, Frey and Giannasca, 1999: p. 173; Giannasca, Giannasca, Falk, Gordon and Neutra, 1994).

Intestinal epithelial cells also sample antigens (Hershberg and Mayer, 2000). Their antigen processing has been described as "complex" and distinct from that provided by M-cells (Hershberg and Mayer, 2000). Again, the function of such presentation is unknown. It has been noted that intestinal epithelial cells in response to non-pathogenic microbes change the status of $CD14^{high}$ to $CD14^{low}$ to increase tolerance of commensals (Haller, Serrant, Peruisseau, Bode, Hammes, Schiffrin and Blum, 2002). However, it is not clear whether these intestinal epithelial cells did not include intraepithelial dendritic cells.

It is thus possible that the principles described here are employed with different varieties of mucosal sampling to refine the identification pathogens and commensals or gain other information. For example, M-cells found outside Peyer's patches might pick up different kinds of pathogen or detect different patterns of pathogen attack.

FUNCTIONAL LEVEL: INFORMATION PROCESSING

Finding a microbe does not positively identify it as a pathogen or a commensal. Indeed, due to the greater presence of commensals in the gut flora, commensal microbes are likely to be found in both samples, albeit, slightly more often in the biofilm than then M-cell one. But this sampling does provide the information that the microbial antigen came from a sample with a bias to have greater or lesser likelihood of being a pathogen. Thus, if a type of microbe is more common in a sample with a bias to pathogens than one without this bias, then it is more likely to be a pathogen rather than a commensal. This information can—if there is appropriate organization of lymphocyte antigen presentation (described below)—be used to pathogen/commensal classify the microbe.

Winner-take-all antigen presentation

For antigen presentation to differentiate pathogens from commensals requires that there exist processes that compare the relative presence of an individual type of microbe in both the M-cell and biofilm derived samples. One way of doing this is to use the output of the two samples as inputs into a second stage involving a winner-takes-all categorization competition (Indiveri, 1997). If the question is whether a microbe is a pathogen, then the input from the M-cells is assigned a positive or activating moiety, and that from the biofilm, a negative or inhibitory one. If more activating inputs exist than inhibiting ones, the winning output will be an activation and the system will, in effect, judge that the input came from a pathogen. If the question is whether a microbe is a commensal, then the input from the M-cells is negative or inhibitory and that from the biofilm positive or activating. In this way, statistical differences can be created between the two samples by biological means that yield through lymphocyte interactions a classification. Neural works have been created that convert bias information from inputs in this winner-takes-all way into categorical output (Hahnloser, Sarpeshkar, Mahowald, Douglas and Seung, 2000; Indiveri, 1997). It is suggested here that this also occurs in gut immunity.

One system requirement will be to adjust the two samples so that the activation/inhibition play off is optimally sensitive. The two samples might vary widely in the quantity of antigens they catch, as a result the activation/inhibition given to them will have to be adjusted to ensure balance. There might need to be the detection of a "not decided" category that could be used to feedback upon sampling so that more M-cells were created or more dendritic cells made journeys across the epithelium to improve the quality of the input information. The activation/inhibition adjustment given to the inputs might also need to be changed in regards to inflammation signals from cytokines and toll-like receptor activation pathways. The classification of an unknown microbe at a time of inflammation, it would be reasonable to suppose, needs the process to allow a lower threshold for M-cell input to activate it as being a pathogen.

Another factor is consistency of antigen sampling. Commensals are long time residents in the gut, while most pathogens are infections picked up from encounters made by the host with conspecies and food sources. There are a number of possibilities here. Antigens from one sample source could be converted into two samples, tonic and transient, and so create temporal derivatives. For example, antigens could be put into a long term buffered "tonic" presentation, and separately, a constantly refreshed, "transient" presentation. These two would provide inputs that would allow for the changing presence of antigen to be detected. An antigen that suddenly appeared is more likely to come from a pathogen than a commensal, while a constantly caught antigen is more likely to be a commensal. Such a "tonic" /"transient" comparison system would need to be adjusted for overall presentation of microbial antigens across time. Another possibility is that the input from M-cell and biofilm sources are initially processed in this way, and that the output from them is then compared. In this case, what would detected would not be the greater proportion of a microbes caught in M-cells compared to in the biofilm but the greater 'spike' in its sampling.

Types of winner-take-all competition

A winner-takes-all set up can be symmetrically (if the output is not activated in way, it is positive in the other), or asymmetrical (one output gets activation, but it might be left undecided whether the alternative is or not present). Whether symmetry or asymmetry is set up depends upon the problem that the system is being asked. This is because different answers have different costs if incorrect. For instance, it may be critical that every microbe that is a pathogen is correctly identified as such, while it may not matter so much that every commensal is correctly judged as a commensal. A few commensals misclassified as pathogens may do no harm, but a

pathogen that is missed because it is misclassified as commensal might endanger the host's survival. In this case, the importance of classifying commensals correctly is secondary to that of classifying pathogens, and the system may be set up asymmetrically to pick pathogens with the result that if the information for this is insufficient that it will not identify commensals.

PHYSIOLOGICAL LEVEL: INFORMATION PROCESSING

(Working out the details of T cell and B cell network interactions is still work in progress so the following comments are highly provisional and incomplete. There are a large number of mucosal T cell subsets and B cells vary in their subsets and their T cell dependence and independence but their functions are either unknown, poorly understood or controversial.)

The immune system is an information processing system as much as the central or peripheral nervous systems. Does it contain the components that could perform the information processing computations required of a winners-takes-all competition?

Information transmission processes

Unlike the nervous system which is based upon fixed connections, information processing in the immune system is based upon cognate matching in which mobile cells associate through MHC presented epitopes and corresponding TCRs or BCRs. This process on T cells takes place before the formation of the immunological synapse (Lee, Holdorf, Dustin, Chan, Allen and Shaw, 2002), and engages two cells in complex presynapse forming information processing that includes activating and inhibitory cofactors. Cell to cell contact communication in the immune system can also involve not only the surface membrane receptors but "directed secretion of cytokines" (Kupfer, Monks and Kupfer, 1994). Physical contact, moreover, between cells can occur over large distances through cell protrusions of up to 50 µm (for example when dendritic cells traffic peptide-MHC complexes to T cells) (Boes et al., 2002).

Information processing inhibition/ activating interactions

Contact between cells of a competitive nature occur at several stages in immune development and activation. They can also happen in several ways. High affinity T cells, for example, can out compete low affinity ones by competitively draining dendritic cells of their antigen (Kedl, Schaefer, Kappler and Marrack, 2002), or homeostatic factors (Barthlott, Kassiotis and Stockinger, 2003). While the cognate interactions are often seen as a single cell to cell contacts, several cells can participate not only as peers but as part of concomitant or sequential orchestrated information processing pathway (for example, between memory and naïve T cells and dendritic cells, Alpan, Bachelder, Isil, Arnheiter and Matzinger, 2004; Leon, Perez, Lage and Carneiro, 2001; or T helper and T killer cells and dendritic cells, Ridge, Di Rosa and Matzinger, 1998).

Output mapping upon immune responses

The winner-takes-all competition requires that dendritic cells derived from M-cell or biofilm sources can be linked to an inhibitory or activating effect upon T cells or B cells. Such effects could be done by dendritic cells of different subsets and so possessing different identifying receptors. The inhibition or activation influence could be arranged in several ways. One possibility is that dendritic cells inhibit T cells using the inhibitory receptor CTLA-4 at time of antigen presentation (Walunas et al., 1994). Also B cell receptors are complexes that are regulated by accessory co-receptors on the B cell surface some of which are inhibitory (Nitschke and Tsubata, 2004).

One assumption here is that antigens can be labeled as to their origin by their dendritic cell subset. This is plausible. Dendritic cells in the mucosal immune system associated with the Peyer's patches are known to exist in several subset types that are distinguished by different co-receptors and co-stimulatory factors and different origins of sampling (Iwasaki and Kelsall, 2001). Dendritic cells have been "associated with M-cells within the M-cell pocket" (Iwasaki and Kelsall, 2001: p. 4889). Myoung-ho Jang and colleagues at the 12th International Congress of Immunology (2004, July 18th -23^{ed} Montreal, abstract no 2600) report two kinds of dendritic cell that express unique combinations of activation markers and adhesion molecules. They note that "DCs bearing a similar phenotype are apparently absent in the Peyer's patches, lymph nodes and spleen". It is thus reasonable to propose that dendritic cells receiving microbes and antigens from M-cells possess different receptors to sub-and intraepithelial dendritic cells.

PHYSIOLOGICAL LEVEL: PATHOGEN DEFENSE AND COMMENSAL MANAGEMENT

The immune system needs to distinguish pathogens from commensals for two separate immunological purposes: pathogen defense and commensal management. These can be expected to differ in their tolerance for classificatory errors, and so how they are set up as information processing systems. Pathogen defense depends upon an efficient detection of pathogens, and therefore is only secondarily concerned with the detection of commensals. A host that misclassified a pathogen as a commensal would threaten its survival while the misclassification of a commensal as a pathogen would only waste immune resources. As a result, pathogen defense will be biased to avoid false negatives (pathogens misclassified as commensals), even if this is at the cost of increased false positives (commensals misclassified as pathogens). Of course, constant misclassification of commensals as pathogens will cause inflammation.

Defense against pathogens will be mainly cellular since pathogens will usually be physically present allowing phagocytosis and other T cell mediated T_H1 responses. As a result, there will be a need for an active suppression of T cells that identify commensal antigens.

The second function of the immune system is commensal management. Commensals exist in biofilms made of mucus and IgA: as such they are not in direct contact with the epithelium which is protected by innate immunity. Here the problem is to identify productive types of commensals. The concern of commensal management will be to link the identification of commensals with information in regard to the benefits coming from specific kinds of commensals, and so allow the direction of targeted support to them (for example, by increasing or decreasing secretory IgA and mucus that aids the formation of the biofilm). Though rarely discussed in mucosal immunology, the epithelium contains sensory cells that are similar in form and possibly function to taste cells (Holzer, Michl, Danzer, Jocic, Schicho and Lippe, 2001). These monitor the chemical nature of the mucus adjacent to the epithelium. Part of their function could be monitoring the health and productivity of the biofilm. To function optimally, commensal management will need to integrate such information with that from obtained from antigens (such as how many commensals are growing in the biofilm as inferred from the amount of antigen fished from the biofilm).

Unlike pathogen defense, commensals management will be largely confined to the colon as that is where most commensals are located.

The commensal management pathway employs specific and nonspecific secretory IgA. This IgA has a multiple uses in commensal management: (1) providing a substrate with mucin for the commensals to form epithelium related biofilm (Palestrant, Holzknecht, Collins, Parker, Miller and Bollinger, 2004; Sonnenburg, Angenent and Gordon, 2004); (2) aggregating microbes to enable elimination, and (3) limiting commensal overgrowth.

Commensal management does not involve cellular responses as commensals are located offshore of the epithelium and so are managed through specific and nonspecific secreted IgA antibodies. As a result of this T cells will not need to be inhibited by T_{reg} cells. Thus, commensal management will have some of the characteristics of a T_H2 immunological response. One possibility is that part of commensal management is microbial “weeding” – that is detecting nonproductive microbes and eliminating them to aid the growth of more beneficial commensals.

Controversy, it should be noted exists, as to whether commensal targeted IgA is regulated by T cell-independent B1 cells (Macpherson, Gatto, Sainsbury, Harriman, Hengartner and Zinkernagel, 2000), or T cell dependent B2 cells (Bos, Jiang and Cebra, 2001; Thurnheer, Zuercher, Cebra and Bos, 2003).

Commensal management is a more complex topic than can be discussed here: the point is that commensal management has very different information processing concerns to that of pathogen defense.

PHYSIOLOGICAL LEVEL: PATHOGEN/COMMENSAL RECOGNITION DYSFUNCTION

Two kinds of mucosal dysfunction (Crohn’s disease and ulcerative colitis) exist that both involve responding to commensals as if they were pathogens: (Bouma and Strober, 2003; Mahida and Rolfe, 2004). Crohn's disease has characteristics of a dysfunction in the pathogen commensal recognition process in pathogen defense, and ulcerative colitis has characteristics of such a defect in commensal management.

Crohn's disease

- inflammation along the whole gastrointestinal tract.
- Inflammation concentrated in some areas more than others and involves nonsuperficial as well as superficial layers (deep ulcers with normal lining between these ulcers).
- Involves T cells mediated response.

Ulcerative colitis

- Inflammation restricted to gastrointestinal tract areas where there are high densities of commensals (the colon and the rectum).
- Inflammation affects all the lining in intestinal compartment in its superficial layers.
- Predominantly an antibody response, though not mucosal IgA but peripheral IgG1 (Thoree et al., 2002).
- Involves activated nonclassical NK T cells (Fuss et al., 2004).

The present paradigms about inflammatory bowl disease assume that it is due either to excessive effector T-cell function or deficient regulatory T-cell function (Bouma and Strober, 2003; Mahida and Rolfe, 2004).

The alternative possibility that can be raised in the context of the above review is that the etiology of Crohn's disease and ulcerative colitis might include a dysfunction in sampling based nonmolecular processes involved in pathogen commensal discrimination with the former one related to detecting pathogens, and the latter for the management of commensals.

ENDNOTE 1

Segmented filamentous bacteria are commensals that attach to the epithelium during weaning with the cooperation of the epithelium cell through means of a nipple-like holdfast segment attachment (Davis and Savage, 1974). Cooperation is also indicated by the provision to the bacteria of "some nutritional factors" (Davis and Savage, 1974: p.955). However, in the absence of specific IgA (Suzuki, Meek, Doi, Muramatsu, Chiba, Honjo and Fagarasan, 2004) segmented filamentous bacteria vastly expand suggesting this cooperation is based also upon active host antagonistic control. It has also been suggested that segmented filamentous bacteria competitively prevent pathogens such as *Salmonella* from colonizing the gut (Heczko, Abe and Finlay, 2000). Segmented filamentous bacteria provide what has been described as "one of the single most potent microbial stimuli of the gut mucosal immune system" (Talham, Jiang, Bos and Cebra, 1999). How do they fit into the above conjecture? I suggest that segmented filamentous bacteria tend to avoid M-cells and prefer to attach to the brush border of epithelial cells. This would cause them to be weighed more greatly in the biofilm sample rather than the M-cell one. Their adjacency to the epithelium could cause them to be constantly over sampled causing them to constantly stimulate gut mucosal immunity.

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