How can the MHC mediate social odor via the microbiota community? A deep dive into mechanisms

Abbreviated title: MHC- and microbiota-mediated social odors

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Funding

This work was supported by the German Research Foundation (project number 416495992 to NS, project number 316099922, 396780709 to JCW); Leverhulme International Fellowship (project number IAF-2018-006 to HJN); and the Alexander von Humboldt Foundation (Humboldt Research Fellowship for Experienced Researchers to HJN).

Acknowledgements

We thank Jim Kaufman, Barbara Caspers, Marc Gilles and Alfredo Sánchez-Tójar for valuable comments.

Competing interests

We declare we have no competing interests.

Authors' contributions

NS and JW conceived the review and designed the figures. NS conducted the literature review and drafted the manuscript. NS, JW and HN revised the manuscript and approved the final version for publication.

Lay abstract

Determining relatedness in members of the same species through their smell can help animals cooperate with close relatives or avoid inbreeding. How genetic information is encoded in odor, and what role immune genes (MHC) and microbes play in generating odor, as well as how they interact is unclear. We outline the immune system's involvement in odor-production, highlight gaps in our knowledge regarding immune gene and microbe-mediated social communication, and suggest ways to advance our understanding.

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Abstract

Genes of the major histocompatibility complex (MHC) have long been linked to odor signaling and recently researchers' attention has focused on MHC structuring of microbial communities and how this may in turn impact odor. However, understanding of the mechanisms through which the MHC could affect the microbiota to produce a chemical signal that is both reliable and strong enough to ensure unambiguous transmission of behaviorally important information remains poor. This is largely because empirical studies are rare, predictions are unclear, and the underlying immunological mechanisms governing MHC-microbiota interactions are

often neglected. Here we review the immunological processes involving MHC class II (MHC-II) that could affect the commensal community. Focusing on immunological and medical research, we provide background knowledge for non-immunologists by describing key players within the vertebrate immune system relating to MHC-II molecules (which present extracellular-derived peptides, and thus interact with extracellular commensal microbes). We then systematically review the literature investigating MHC-odor-microbiota interactions in animals and identify areas for future research. These insights will help to design studies that are able to explore the role of MHC-II and the microbiota in the behavior of wild populations in their natural environment and consequently propel this research area forward.

KEYWORDS: Major histocompatibility complex, scent, tolerance, kin recognition, immune response, systematic review

Introduction

Animals use olfactory cues during social communication, and microbiota have been implicated in governing chemical cues relevant for social communication (Archie and Theis 2011; Maraci et al. 2018). Furthermore, genetic determination of the microbiota's composition (Zoetendal et al. 2001; Stewart et al. 2005) and its shaping by the host immune system, specifically the major histocompatibility complex (MHC) (Toivanen et al. 2001; Kubinak et al. 2015; Wadud Khan et al. 2019), have been hypothesized and investigated. However, the number of empirical studies is limited, and they often neglect the underlying immunological mechanisms linking microbiota and odor, and therefore do not allow the formulation of clear predictions for testing. Thus, the purpose of this review is to summarize the extensive medical and immunological literature linking the key players potentially involved in generating microbial-based odor cues for social communication and to present immunological evidence that could aid in prospective study design and interpretation of results. We first introduce links between the MHC, microbiota, and odor signaling. We then present the state of knowledge of the immunological mechanisms governing host microbial communities. Finally, we systematically review empirical studies investigating MHC-microbiota-odor associations to identify areas in need of future research.

Odor and social communication

Animals use olfactory cues, such as scent marks or body odor, to broadcast information. In mammals, scent marks include secretions from anal, genital, frontal, or sternal glands, as well as urine and feces (Johnson 1973). Birds can perform "bill-wiping" to mark substrates with secretions from their uropygial gland (Whittaker et al.

2014). Similarly, fecal pellets (Gautier and Miaud 2003) and post-cloacal gland secretions (Simons et al. 1994) in amphibians and femoral gland secretions in reptiles (Mason and Parker 2010) can act as scent marks. These secretions appear to play an important role in social communication (Johnson 1973) and there is evidence that scent marks and body odor, which is generated by secretions and metabolites remaining on the body, provide a wealth of information about the dispatcher.

Chemical signals can transfer information about an individual's status (such as sex, age, rank and sexual receptivity (Greene and Drea 2014; Harris et al. 2014; Vaglio et al. 2016; Marneweck et al. 2017; Spence-Aizenberg et al. 2018)) to conspecifics. Similarly, information on general health (Harris et al. 2018), parasite load (Mitchell et al. 2017), or infection and injury (Zala et al. 2004) can be conveyed through scent. This may occur through particular chemicals associated with the infection or the immune response to it (for example Arakawa et al. 2010), or through reallocation of resources or the presence of fever affecting the microbial community (Harris et al. 2018). Signature mixtures (variable mixtures of chemicals) can be used for individual and social group recognition (Smith 2006; Scordato et al. 2007; Theis et al. 2012; Theis et al. 2013), and to assess relatedness and genetic compatibility (Charpentier et al. 2008; Stoffel et al. 2015).

Usage of such chemical signals can have important fitness consequences as identifying relatives helps to avoid inbreeding depression (Pusey and Wolf 1996) and enables help to be directed towards close relatives, increasing indirect fitness (Hamilton 1964). Apart from determining relatedness, odor might be used to perceive genetic quality of a potential mate (in terms of "good genes" or genetic diversity), and genetic compatibility, which can be independent of overall relatedness (Lenz et al. 2009). This may in turn increase genetic quality and thus offspring attractiveness or

survival, resulting in elevated parental fitness (Møller and Alatalo 1999). Both genetic diversity and similarity might be signaled through odor profiles, but assessing similarity requires a self-referencing mechanism for comparing conspecifics' to an individual's own odor (Hauber and Sherman 2001).

Odors providing information on the genetic make-up of an individual, such as relatedness, quality, and compatibility, are particularly interesting as their nature suggests that they must have a genetic basis. An excellent candidate exhibiting sufficient polymorphism for conveying genetic information while also having an important role in immune response are the genes of the MHC.

A promising candidate – the MHC

The MHC encodes membrane glycoproteins essential for the adaptive immune response (Bjorkman et al. 1987) through regulating discrimination between self-derived and foreign peptides, and is present across jawed vertebrates (Kaufman 2018). The MHC molecules bind peptides and present them to professional immune cells, which then either initiate immune response or not (Knapp 2005). MHC molecules are divided into class I and II, with class I molecules (MHC-I) being expressed on nearly all nucleated cells. They present peptides mostly from the cytoplasm to cytotoxic T cells which, once activated, can initiate the death of the MHC-peptide carrying cell (Klein 1986). In contrast, class II (MHC-II) molecules are expressed by professional antigen-presenting cells (APCs) (e.g. macrophages, B cells and dendritic cells, among others), and present engulfed peptides (Neefjes et al. 2011). Therefore, MHC-I mostly presents self-derived peptides and peptides originating from viruses or other pathogens that have entered the cell, while MHC-II molecules predominantly present peptides derived from exogenous sources, such as

bacteria or parasites, that have been ingested by the MHC-II carrying cell (Rammensee et al. 2013). Throughout we refer only to classical MHC, distinguished from nonclassical by solely presenting peptides to T cells and having high expression and polymorphism (Braud et al. 1999; Alfonso and Karlsson 2000). Instead, functions of nonclassical MHC are diverse, including antigen processing and immunomodulatory effects in both innate and adaptive response (Braud et al. 1999; Alfonso and Karlsson 2000).

Both classical MHC-I and -II molecules have high polymorphism that is most pronounced in the peptide binding region that contains the peptide binding sites (PBS) interacting directly with the antigen (Bjorkman et al. 1987; Brown et al. 1993). This polymorphism enables presentation of a wide range of peptides, with greater functional difference between alleles, encoding for different PBS, leading to a greater number of peptides bound (Pierini and Lenz 2018). Hence, individuals expressing many different MHC molecules should theoretically be able to detect a higher variety of peptides and thus interact with a greater range of microbes which might in turn be reflected in their odor.

An army of supporters - the commensal microbial community

Animals host a diverse range of microbial phyla on their surfaces such as the skin, glands and gut (Ley et al. 2008). Before birth or hatching, mammals, birds and reptiles reside in environments classically considered sterile, although this view is questioned (Kohl 2012; Perez-Muñoz et al. 2017; Trevelline et al. 2018). After birth or hatching, animals acquire bacteria from their surrounding environment, including the mother's birth canal and genitalia during birth, as well as from parents, litter or nest mates (Kohl 2012; Sylvain and Derome 2017). Successive colonization events result

in composition shifts until a rather stable commensal population has formed (Luckey 1972; Kohl 2012; Oh et al. 2012).

Interestingly, microbiota composition can differ considerably between individuals of the same species (Jami and Mizrahi 2012). These inter-individual differences can be related to exogenous factors, such as stochastic microbe population dynamics, diet and environment (reviewed in Spor et al. 2011; Davenport et al. 2014; Rothschild et al. 2018). Additionally, endogenous factors, such as an animal's stage of life, the body site's microclimate, and the host's genotype can influence an individual's microbiota (Spor et al. 2011). The microbial community appears to display a certain stability and dependence on host genetics, as it can re-establish even after severe perturbation such as antibiotic treatment (for example Antonopoulos et al. 2009). However, evidence from human twin studies investigating the microbiota's genetic basis is ambiguous with some claiming genetic determination (for example Stewart et al. 2005; Goodrich et al. 2014) while others do not support this dependency (for example Turnbaugh et al. 2009).

Hosting microbiota can provide fitness benefits, such as disease resistance (Rosshart et al. 2017) and metabolic efficiency (Tremaroli and Bäckhed 2012), causing the host's immune system to face a conflict: ensuring clearance of harmful pathogens while simultaneously tolerating beneficial commensals. Disruption of this balance can spark dysregulated or overaggressive immune responses towards harmless materials resulting in persistent inflammations or autoimmune diseases (Chung and Kasper 2010). Hosting microbiota may also help signal information used in social communication (Archie and Theis 2011). Albone and Perry (1974) proposed the fermentation hypothesis stating that microbes inhabiting bodily surfaces produce substances detectable by conspecifics. Regulation by immune genes, such as those

of the MHC, may therefore cause microbiota to reflect their host's genetic composition (Khan et al. 2019).

MHC involvement in odor production

The MHC may directly affect odor by either binding non-volatile peptides acting as a source of odor (peptide hypothesis) (for example Milinski et al. 2005; Spehr et al. 2006; Hinz et al. 2013; Milinski et al. 2013), or less likely, through MHC molecules themselves breaking down to become odorants (MHC molecule hypothesis) (Boehm and Zufall 2006). MHC molecules might also indirectly affect odor in two ways (Fig. 1). First, MHC molecules, as key players in the immune response, have the potential to affect the outcome of infections with viruses or parasites thus affecting the health status of an individual, which can be reflected in volatile composition of odor (Kimball et al. 2013; Grieves et al. 2018). Second, MHC molecules might affect odor through regulating the composition of the commensal flora (microflora hypothesis) (Singh et al. 1990). Specifically, these commensal microbes produce volatiles as products of their metabolism and thus influence odor. Due to the MHC's polymorphism and its central role in the adaptive immune response combined with the diversity of microbial species, regulation of microbially-produced odor cues via the MHC has the potential to generate detailed cues for social communication and thus we decided to further elaborate on this interaction.

Control of the microbiota by the MHC might happen via different mechanisms that can also be of direct and indirect mode. The MHC might govern microbiota directly by binding and presenting peptides and thus inducing an immune response aimed at the peptide source (Howard 1977; further details are given in the paragraph below on the activation of T cells). Alternatively, the MHC might shape microbiota indirectly and

there are several hypotheses describing the mechanism of such an indirect link. As supposed by the peptide-microbe hypothesis, the MHC allele-specific immune responses might affect what molecules are available to the microbiota to metabolize thus influencing microbiota composition and consequently microbially produced odors. Because immune responses are mounted against microbial peptides matching the PBS of the MHC molecule, MHC allele diversity might determine the repertoire of peptide ligands that is available to the microbial community to metabolize. Furthermore, by immunologically controlling microbiota composition, MHC allele diversity might govern molecules and microbial secondary metabolites available to the microbes, the products of which might affect odor (Penn and Potts 1998a). Alternatively, regulation by the MHC might cause inter-specific interactions between microbes and thus indirectly determine microbiota composition by favoring or preventing the establishment of certain species. Additionally, the MHC can influence other adaptive immune mechanisms following peptide detection via the MHC that lead to tolerance towards certain microbiota species (Kubinak et al. 2015; Khan et al. 2019; see also the paragraph on the role of IgA).

Individuals might discriminate MHC-based microbial odor using a familial imprinting system and thus base their mate choice decisions on learned familiarity cues as observed in mice (Yamazaki et al. 1988). In a more elaborate mechanism called self-referencing, individuals use their own odor as a reference for comparison of conspecific odors to optimize offspring genetics (Reusch et al. 2001; Aeschlimann et al. 2003; Milinski et al. 2005).

The underlying chemical properties of the molecules suspected to carry information via direct or indirect mechanisms of MHC-linked odor signaling differ substantially (see Penn and Potts 1998a; Ruff et al. 2012; and Overath et al. 2014 for critical discussion of the mechanisms). Both the peptides bound by MHC molecules as well

as the MHC molecules themselves, which are supposed to serve as odorants, are non-volatile peptides. Despite their non-volatility, there is strong evidence for MHC peptide ligands to convey information about the MHC. Female sticklebacks have been shown to use a self-referencing mechanism and count alleles of their potential mates to optimize their offspring's MHC composition (Reusch et al. 2001; Aeschlimann et al. 2003). In a further experiment Milinski et al. (2005) determined the source of information used by the female sticklebacks by experimentally modifying the odor of males with synthetic MHC peptide ligands. Thus, it is possible for MHC genotype to be detected without the involvement of the microbiome. However, non-volatile peptides are unlikely to be the only indicators of MHC genotype as the urine of MHC-congenic mice devoid of peptides could still be discriminated (Singer et al. 1993; Kwak et al. 2009). This suggests that volatile molecules produced by the bacterial metabolism might generate MHC-based odors as well. In addition, while MHC-dependent peptide ligands corresponding to different MHC molecules can evoke unique activation patterns reflecting MHC composition (Leinders-Zufall et al. 2004), many MHC molecules can bind the same set of peptides. For example, up to 50% of peptide ligands bind multiple MHC-I molecules in humans (Rao et al. 2011). Overlap in MHC-mediated activation patterns would prevent unambiguous sensory discrimination of MHC composition suggesting that additional information may be required to reliably determine MHC genotype via odor.

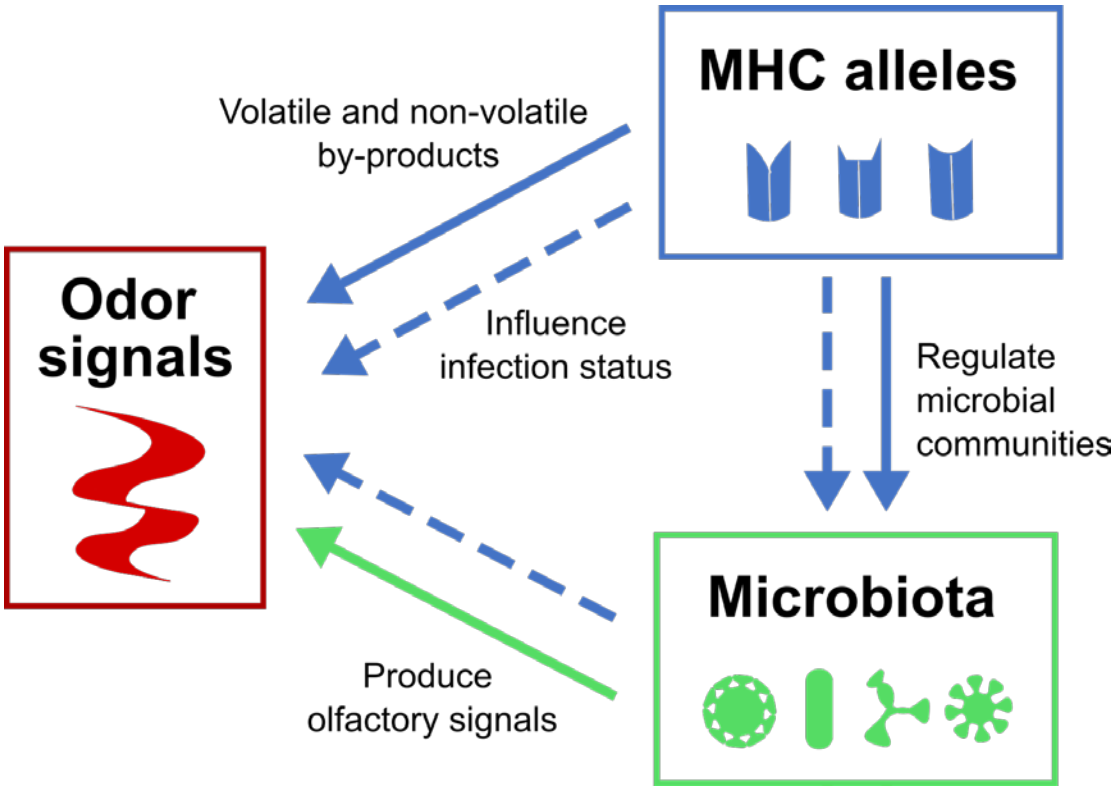


Figure 1. MHC-microbiota interactions in chemical communication. Schematic of the interactions between genes of the MHC and the microbiota and their potential influence on odor. MHC polymorphism (blue arrows) might directly influence odor (solid arrows) through volatile and non-volatile by-products such as urinary signals or peptide ligands or indirectly (dashed arrows) by influencing infection status or through regulation of the microbiota (green arrow) producing volatiles.

Potential MHC-related mechanisms of microbiota structuring

With its immunological function and high polymorphism, the MHC rightly is a promising candidate for governing microbially-derived odor cues. However, still many questions remain unanswered. For example: How does a system evolved to eliminate pathogens establish tolerance to microorganisms? How does the MHC

orchestrate microbiota composition and maintain its stability? How does MHC diversity affect microbiota composition?

Knowledge of the immunological mechanisms of MHC-microbiota interaction poses the basis for establishing hypotheses and for the interpretation and validation of results, and four conflicting predictions of the relationship between MHC and microbial diversity have been made. One possibility is a negative correlation between MHC diversity and microbiota diversity (Bolnick et al. 2014; Leclaire et al. 2019). Considering the MHC's role in the response to pathogens and that each MHC molecule binds a particular repertoire of peptides, a higher diversity of MHC molecules might lead to a higher diversity of peptides presented and thus a larger number of microbes that can be eliminated, causing lower microbiota diversity (Fig. 2A). Second, it is possible that we may observe the reverse relationship, with higher MHC-II diversity causing higher microbiota diversity (Hernández-Gómez et al. 2018). This is possible because the immune system does not only eliminate microbes but also forms symbiotic bonds with commensals, hence a positive correlation may arise if a higher diversity of MHC molecules initiates tolerance to a more diverse range of microbes (Fig. 2B). Consequently, both negative and positive relationships signaled via the microbiota should theoretically enable detection of MHC diversity. Third, certain MHC motifs might also interact with specific groups of microbes, leading to covariation of MHC genotypes with specific microbial community structuring (Fig. 2C). This association of certain MHC alleles with particular microbes could allow the detection of specific alleles and thus enable choosing a mate with complementary MHC alleles via self-referencing. Finally, MHC and microbiota diversity or composition may not be linked, as genes other than the MHC or environmental influences might determine the commensal community of a host (Fig. 2D). Indeed, the specificity between MHC genotype and microbiota community should not be

assumed a-priori. The great variety of microbial species and microbial peptides derived from each species results in a plethora of different peptides that can act as ligands for MHC molecules. Hence it is possible that the great diversity in MHC ligands impedes specificity of MHC-II-bound microbes (Rammensee et al. 1999).

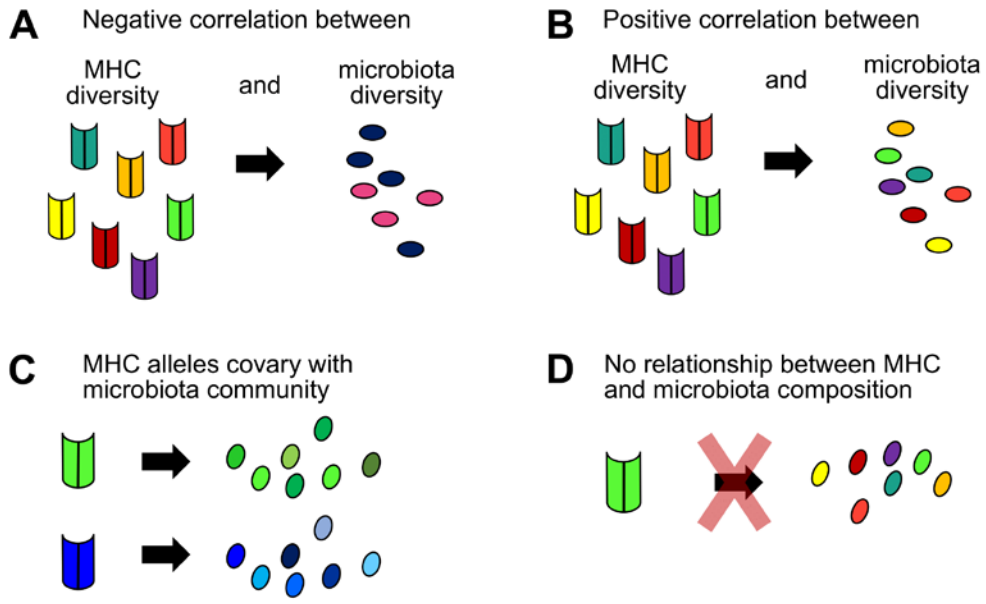


Figure 2. MHC-microbiota interaction. (A) A negative correlation is characterized by high MHC diversity leading to low microbiota diversity. (B) A positive correlation is caused by high MHC diversity tolerating more diverse microbiota communities. (C) Covariation between MHC genotypes and microbiota community structure is caused by specific MHC binding motifs selecting for the presence of certain groups of microbes. (D) No detectable relationship between MHC and microbiota community indicates the MHC is not a major determinant of the microbiota community.

MHC-microbiota interactions will also be affected by the diverse habitats that microbes experience on different host surfaces. A recent meta-analysis investigating

363 the association of environmental and host physiological and phylogenetic factors with
364 the microbiome indicates that external microbiomes, such as skin or feather
365 microbiomes, are best explained by environmental factors such as precipitation
366 seasonality and temperature (Woodhams et al. 2020). In contrast, internal
367 microbiomes derived from feces or the gut, were best explained by host associated
368 factors such as immune complexity/phylogeny, trophic level or diet, and climate.
369 Moreover, within the same host or even organ, body site-specific microclimates
370 cause varying local microbial communities (Spor et al. 2011), and tissue-specific
371 immunological adaptations limiting inflammation and increasing tolerance to
372 microbes exist. Nonetheless, different organs such as the skin and the gut also show
373 major histological and immunological commonalities (Artis 2008; Pasparakis et al.
374 2014). Both organs have an epithelia-cover, rely on immune response initiated by
375 MHC-II-bearing cells and share tolerance-facilitating components (Hepworth et al.
376 2013; Kobayashi et al. 2019). Hence, the relationship between MHC-II and the
377 microbiota should theoretically apply similarly to different organs. However,
378 understanding of the immunological crosstalk between the microbiota and tissues
379 remains limited.

380

381 **Understanding the immunological mechanisms – what we know so far**

382 Understanding the causal connections between the MHC and the microbiota might
383 reveal new questions and solve existing challenges in diverse fields. Hence, we now
384 provide an overview of MHC-related mechanisms initiating either an immune
385 response or tolerance of microbiota. Specifically, we review findings from
386 immunology and medical research, particularly in mice and humans, where the
387 interplay between the immune system and commensal bacteria has been extensively

researched. However, we do not aim at explaining these immunological processes in their great complexity and detail but rather focus on the mechanisms involving the MHC and the microbiota (for further review, see Marietta et al. 2015; Honda and Littman 2016). We want to provide immunological background knowledge on the interrelation of the MHC and the microbiota potentially important for chemical communication for a non-immunologist audience to help explain the observed patterns of MHC and microbiota correlation and covariation in empirical studies.

We note that there are reports of the MHC, particularly MHC-I, directly influencing odor either through the MHC molecules itself or its peptide ligands acting as odor cues (for example Leinders-Zufall et al. 2004). Nonetheless, as we want to summarize findings that help understand the possible interactions of the MHC with microorganisms as a potential regulator of odor, we focus only on MHC-II because these molecules predominantly present phagocytized antigens originating from extracellular microorganisms, such as commensals.

Starting the fight – or not? Initiating the adaptive immune response

Antigen-presenting cells (APCs), such as B cells or macrophages, phagocytize and process peptides and present them with their MHC-II molecules together with other surface molecules to helper T (Th) cells, a certain type of T (developing in the thymus) cell (Neefjes et al. 2011). The interaction between the APC and the Th cell can either cause an immune response towards the presented antigen (Fig. 3A) or no response (Fig. 3B) (Jurewicz and Stern 2019). Activation of the Th cell only occurs if it can recognize the antigen and thus T cell responses depend on the repertoire of T cell receptors (TCRs) available, which is determined during T cell development and maturation.

413 During T cell development, tolerance to certain antigens is initiated in a two-step
414 process, called positive and negative selection, within the thymus (reviewed in detail
415 in Jurewicz and Stern 2019). During positive selection, T cells are selected for their
416 ability to respond to MHC-self-peptide complexes, with those that do not respond
417 being eliminated (Huseby et al. 2005). The second step, negative selection,
418 describes the elimination of T cell receptors showing an excessive response to MHC-
419 self-peptide complexes (Klein et al. 2014). Thereby, T cells potentially causing
420 autoimmune reactions are excluded. Once outside of the thymus, the remaining T
421 cells receive boosting signals from MHC II-bearing cells which stimulates their
422 survival. Consequently, the diversity of the TCR repertoire together with the MHC-II
423 molecules determines the set of peptides against which an adaptive immune
424 response is mounted. Thus, complementary to the mechanisms by which MHC-II
425 diversity might impact microbiota composition (see also the paragraph on MHC-
426 related microbiota structuring), the TCR diversity has the potential to regulate the
427 commensal microbiota.

428 But how exactly does the MHC's polymorphism influence the TCR repertoire, thus
429 affecting adaptive immune responses and potentially governing microbiota?
430 Theoretical models suggest that MHC diversity can be negatively linked to the TCR
431 repertoire retained after selection in the thymus (Nowak et al. 1992; Woelfing et al.
432 2009). This relationship depends on the higher diversity of MHC molecules leading to
433 more TCRs being removed during negative selection because of self-reactiveness.
434 Thus, individuals should try to achieve an intermediate number of MHC alleles in
435 their offspring to optimize resistance to parasites (Wegner, Reusch, et al. 2003;
436 Wegner, Kalbe, et al. 2003). An empirical study on bank voles (*Myodes glareolus*)
437 supports this negative relationship between MHC diversity and TCR repertoire,

though only for MHC-I and not MHC-II (Migalska et al. 2019). Consequently, the relationship between MHC-II and TCR diversity has not been fully explained.

Apart from the interplay between the TCR and the MHC-II during thymic selection, the type of T cell involved as well as additional signals can influence the outcome of the APC-T cell interaction (Benchareau and Steinman 1998). For naive Th cells that have not encountered the antigen before, activation by the MHC-II-peptide-complex alone does not cause an immune response. Instead, it requires additional costimulation from the APC consisting of an interaction of different receptors present during inflammation to elicit an immune response (the ‘danger signal’). Lack of this second costimulatory signal can thus prevent immune responses towards antigens of non-pathogenic origin (Fig. 3B; Bour-Jordan et al. 2011; Chen and Flies 2013) and facilitate symbiotic relationships with commensals.

Once a Th cell has been activated by an APC through the MHC-II-peptide complex in combination with a costimulatory signal, it can in turn activate other immune cells, such as B cells. This causes B cells to initiate antibody production (Fig. 3A). Furthermore, B cells can bind and internalize free antigens via their B-cell receptor, initiating their maturation and antibody production as well. More frequently than that, B cells act as APCs themselves, presenting peptides via their MHC-II molecules to T cells to initiate activation of further immune cells such as other B cells (Sprent 1984). Consequently, as B cells themselves carry MHC-II molecules and T cells depend on MHC-II-carrying APCs for activation, B cell-T cell interaction as well as antibody production by B cells depend on the allelic polymorphism of MHC-II (Hiinig and Schimpl 1979; Sprent 1984).

Tiny but mighty – IgA performs diverse tasks

After activation by MHC-II-activated Th cells, B cells can produce antibodies, called immunoglobulins of the class A (IgA). This class of antibodies performs diverse tasks and plays an important role in mediating tolerance to commensals on mucous surfaces such as the gut. IgA not only combats viruses, bacteria and toxins through neutralization, agglutination, and binding (Pabst 2012), but is also involved in diminishing inflammatory and oxidative responses towards microbiota and reducing their pathogenicity (Peterson et al. 2007; Cullender et al. 2013). This key role in regulating tolerance is demonstrated in patients with low IgA-levels who suffer from an overactive or misregulated immune response (Ammann and Hong 1971; Teahon et al. 1994).

The interaction between APC, Th cell, and B cell necessary to initiate antibody production depends on the diversity of MHC-II molecules. A more diverse repertoire of MHC-II molecules on APCs enables detection of a wider range of peptides. Consequently, a wider range of peptides recognized by MHC-II molecules interacts with a more diverse set of Th cells and thus results in a more diverse set of activated B cells producing a more diverse set of IgA. In turn, the resulting larger IgA repertoires facilitate tolerance against a wider range of microbes (Fransen et al. 2015). For example, Fransen et al. (2015) demonstrated a positive relationship between IgA diversity and microbiota diversity in two mice strains differing in several immunological features. As similar levels of IgA diversity could not be achieved by cohousing of mice nor by fecal transplants in one strain, they concluded that contact with microbiota alone might not be sufficient to increase IgA diversity and that there might be a genetic basis to the production of diverse IgA. By influencing the IgA repertoire, MHC-II diversity might hence be positively linked to microbiota diversity through facilitating tolerance responses.

Keeping the peace – Treg cells and ILCs

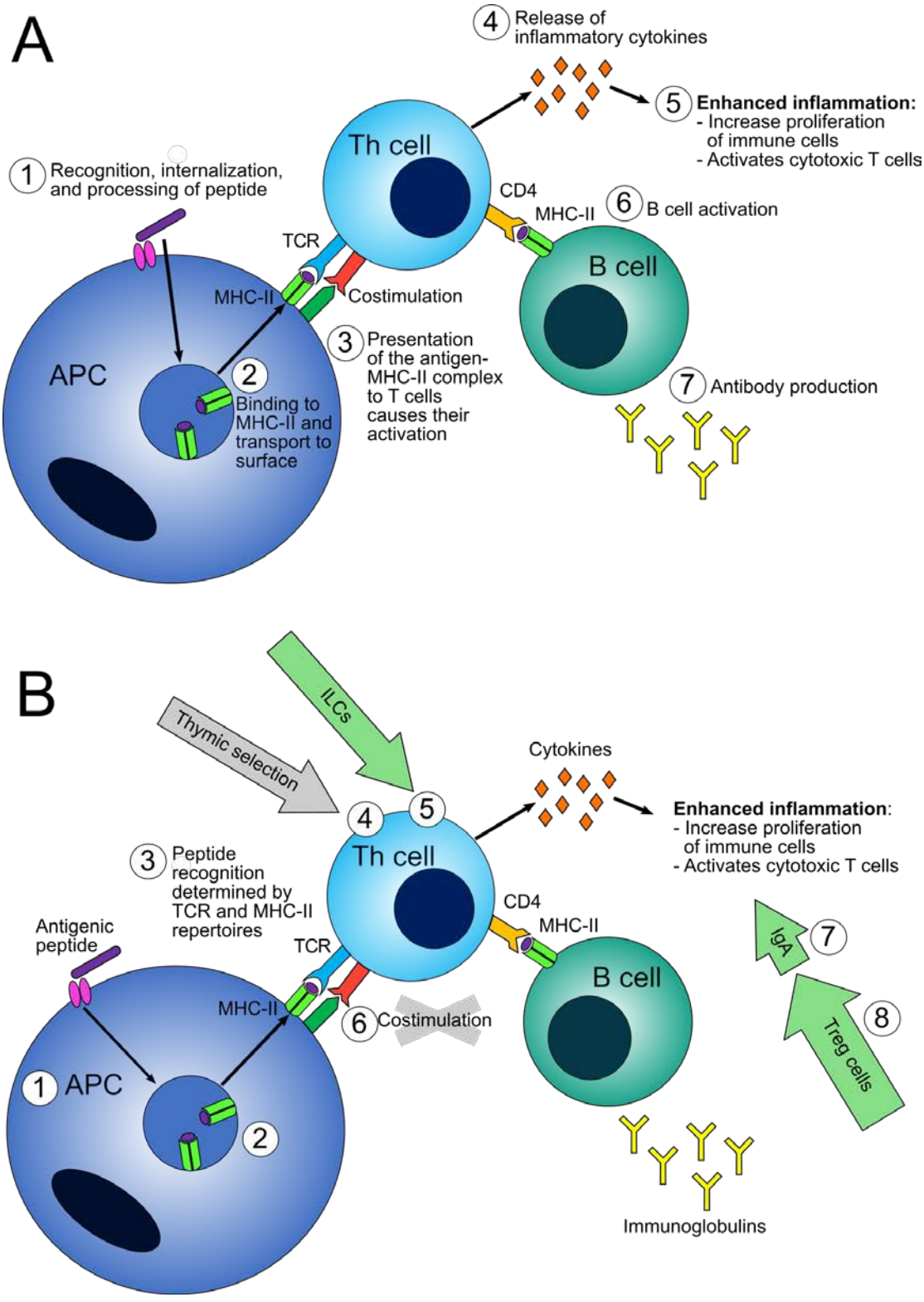
Apart from mounting immune responses aimed at eliminating pathogens, the immune system must be capable of tempering inflammation to protect tissues from oxidative damage, to promote tolerance to benign foreign entities, and to enable symbiotic relationships with commensals. Hence the immune system includes anti-inflammatory components such as regulatory T (Treg) cells (Fontenot et al. 2005) and innate lymphoid cells (ILCs), which are involved in maintaining homeostasis towards commensal microbiota (Hepworth et al. 2013; Hepworth et al. 2015).

Alterations in this anti-inflammatory response can have severe consequences for the immune system and the microbiota. Inhibiting the ability of ILCs to process and present peptides through selective deletion of their surface-bound MHC-II molecules causes a dysregulated immune response towards commensal bacteria and thus facilitates spontaneous intestinal inflammation (Hepworth et al. 2013). These findings indicate an MHC-II-dependent mechanism involving ILCs by which homeostasis is promoted and overreactive immunological responses against commensal microbiota are reduced. Furthermore, ILCs intrinsically expressing MHC-II induce cell death of T cells that act against commensal bacteria thus providing a potential role for the MHC-II to act on microbiota composition through enhancing tolerance (Hepworth et al. 2015).

Similar to the inhibition of ILCs, the loss of specific Treg cells can have consequences for gut homeostasis and involves a decline in IgA levels (Cong et al. 2009), which in turn have an important role in shaping the microbiota community (see previous section). These findings were reinforced by discoveries made by Josefowicz et al. (2012) who created mice deficient in a certain type of Treg cell and thereby caused increased levels of cytokines acting against extracellular parasites paired

514 with mucosa-associated inflammation. Since these mice additionally showed an
515 altered microbiota composition, they concluded that these Treg cells play an
516 important role in orchestrating the composition of the microbiota.

517 For the generation of both Th and Treg cells, microbiota appear to play a crucial role
518 (for example Strauch et al. 2005; Atarashi et al. 2008). Kawamoto et al. (2014) even
519 postulated a symbiotic regulatory loop in which Treg cells modulate microbial
520 diversity by tempering inflammation and facilitating higher IgA diversity (Fig. S5).
521 Likewise, increased microbiota diversity promotes Treg cell diversity and thus IgA
522 diversity. Consequently, as T cell and B cell activation and thus IgA production is
523 linked to MHC-II polymorphism, MHC-II diversity has the potential to influence
524 microbiota composition and diversity via this symbiotic regulatory loop including IgA
525 and Treg cells. MHC-II polymorphism displays potential in attenuating adaptive
526 immune responses and enhancing tolerance towards microbiota. However, despite
527 evidence for the MHC-II initiating and regulating adaptive immune responses aimed
528 at the microbiota, the mechanisms of how exactly MHC-II allelic diversity affects
529 tolerance towards a broader community of microbes has yet to be answered.



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531 Figure 3. Immune response. Steps of immune response involving MHC-II leading to (A)

532 elimination and (B) tolerance of the pathogen. (A) (1) After recognition by an APC, the peptide

533 is internalized, processed and (2) presented by the MHC-II. (3) Interaction of the MHC-II-

peptide-complex with the TCR together with an inflammatory costimulatory signal cause Th cell activation. (4) Inflammation is further exacerbated through cytokine release by Th cells, (5) causing activation of cytotoxic T cells and increased proliferation of immune cells. Activated Th cells (6) activate B cells that (7) produce antibodies. (B) (1) The type of APC as well as (2) the processing of the peptide can influence peptide recognition. (3) MHC-II and TCR strongly affect the set of presented peptides and the type of response. (4) MHC-II diversity is genetically determined, whereas the TCR repertoire is also determined by thymic selection. (5) ILCs can temper inflammation by inducing cell death of T cells acting against commensal bacteria. (6) In case of missing costimulation through an inflammatory signal, Th cell activation is prevented. (7) IgA produced by B cells can facilitate tolerance. (8) Treg cells promote IgA diversity and thus temper inflammation. Arrows displaying processes are colored in grey, cellular or humoral components are colored in green.

Systematic review of the evidence

To investigate the current evidence provided by empirical studies on the mechanisms linking the MHC, microbiota, and odor, we systematically reviewed the literature up to 30th January 2020 in both PubMed and Web of Science. We excluded human studies, as they include cultural, technological, and socioeconomic features unique to humans (reviewed in Winternitz and Abbate 2015), which could influence microbiota, odor, and behavior. Full steps for the systematic review, including search terms, PRISMA flowchart, studies included and excluded, and reasons for exclusions, can be found in the supplementary materials (Tab. S1-S3, Fig. S1-4, supplementary methods).

Overall, we screened 577 publications (from both search engines combined, no duplicates) and retained 64 publications relevant for our review (listed in Table S1-S3). These were subdivided into those on the relationship between the microbiota

and odor (n = 6 studies; Table S1), the MHC and odor (n = 51 studies; Table S2), and the MHC and the microbiota (n = 7 studies; Table S3). We did not find any publication that had investigated the interaction of all three components: MHC, microbiota, and odor.

Through additional searching for relevant publications in recent reviews and publications, we found nine publications (including 3 studies not indexed) that had not been captured by our systematic search. However, we agree with Nakagawa and Lagisz (2019) that comprehensiveness of a systematic review can be impracticable or even impossible to achieve. Instead, requirements of a good systematic review are unbiasedness and transparency in the search process. This can be achieved by conducting the searches in at least two data bases and predefining search and data extraction strategies (Nakagawa et al. 2017). Since we fulfill these prerequisites of best practice, we contend that our systematic search is of appropriate quality and defend the usage of our search strings (to be comprehensive, the nine relevant but missing studies are included in the supplementary methods and labeled as such). Thus, we added nine relevant publications (microbiota and odor: n = 5 publications; MHC and odor: n = 2 publications; MHC and microbiota: n = 3 publications, with one publication (Zomer et al. 2009) found in our search for MHC and odor covering both topics), yielding a total number of 73 relevant publications. In the following sections, we summarize these findings (an extensive list of publications can be found in the supplementary materials, Table S1-S3).

Microbiota and odor

The 11 publications that have investigated potential links between microbiota and odor have been conducted solely on wild species (with the exception of one hybrid; a

585 Bengal cat (*Felis catus* × *Prionailurus bengalensis*)) (Table S1). Support for a
586 relationship between microbes and volatile chemicals that compose odor profiles
587 comes from studies on spotted hyenas (*Crocuta crocuta*) (Theis et al. 2013),
588 European badgers (Buesching et al. 2016), meerkats (*Suricata suricatta*) (Leclaire et
589 al. 2017) and South American tree frogs (*Boana prasina*) (Brunetti et al. 2019), in
590 which odor and microbiota profiles, obtained from secretions from the subcaudal
591 scent pouch or gland, anal glands and skin respectively, showed significant
592 covariation. However, this was not the case in great tits (*Parus major*) (Jacob et al.
593 2018) and Carolina dark-eyed juncos (*Junco hyemalis carolinensis*) (Whittaker et al.
594 2016). Despite missing covariation between odor and microbiota profiles in Carolina
595 dark-eyed-juncos, which might be caused by either only a subset of the microbiota
596 contributing to odor or redundancy in the odor-producing members of the microbial
597 community, the ability of members of the microbiota community to produce volatiles
598 found in secretions has been demonstrated in northern dark-eyed juncos (*Junco*
599 *hyemalis hyemalis*) (Whittaker et al. 2019). Likewise studies on meerkats (Leclaire et
600 al. 2017) and a Bengal cat (Yamaguchi et al. 2019) found microbes associated with
601 volatile production, suggesting that microbes contribute to odor in these species.
602 Evidence for the involvement of bacteria in odor generation also comes from African
603 elephants (*Loxodonta africana*), where Goodwin et al. (2016) showed that removal of
604 bacteria from exogenously aging urine of African Elephants hindered the formation of
605 odorous compounds.

606 Evidence for a causal mechanism linking the microbiota community and odor was
607 found in a study conducted by Whittaker et al. (2019) in which antibiotics were used
608 to artificially perturb the microbiota in northern dark-eyed juncos. This treatment
609 affected the volatile odor profile, which had been linked to the presence of particular
610 bacterial species in a previous experiment on Carolina dark-eyed juncos (Whittaker

et al. 2016). Support for a direct link between microbiota and odor also comes from a comparable study on European hoopoe nestlings (*Upupa epops*) (Martín-Vivaldi et al. 2010) and from Indian mongooses, in which secretions from antibiotically treated anal pockets were observed to lack chemical compounds that are present in secretions of untreated anal pockets (Gorman et al. 1974).

All eleven studies investigated the effect of microbiota on odor by analyzing odor profiles developed using gas chromatographic methods such as gas chromatography – mass spectrometry (GC-MS, a technique that separates odor into its chemical subcomponents based on chemical properties and mass), and studies did not investigate whether chemical differences were detected or responded to by conspecifics. Thus, evidence for the ability of animals to detect these differences in the odor profiles for social communication is still lacking.

MHC and odor

The influence of the MHC on odor has been of particular interest in studies of MHC-dependent mate choice as well as kin discrimination. In this regard, the ability of animals to detect MHC-differences in conspecifics' or other animals' odors has been studied extensively (reviewed in Kwak et al. 2010). In early studies, laboratory animals were trained to differentiate between odors of conspecifics or other laboratory species. Results showed that mice could discriminate between odors of strains differing only at the MHC (Bard et al. 2000; Willse et al. 2006), that MHC-linked odor differences are already detectable in pups (Yamazaki et al. 1992), and that fetal MHC-odortype is discriminable in pregnant mice (Beauchamp et al. 1994). However, these pioneering studies often rely on small sample sizes of laboratory strains using mostly Y-maze odor discrimination trials (Table S2). A criticism of odor

636 discrimination trials is that the ability to discriminate odors could arise due to training,
637 resulting in laboratory animals discriminating cues that their untrained counterparts
638 cannot distinguish in a natural situation (Penn and Potts 1998b). Our literature search
639 found 19 preference trials testing untrained animals (both wild or wild-caught (n = 14)
640 and laboratory (n = 5)) in flow chambers or y-mazes, and these studies
641 predominantly support an important role for MHC-based cues in mate choice or kin
642 recognition (for example Grieves et al. 2019). Importantly, preference trials have
643 since been complimented by habituation/dishabituation trials under naturalistic
644 settings, fortifying evidence for the discriminability of MHC-based odor differences
645 (Brown et al. 1989; Penn and Potts 1998b) with a certain minimum distance at the
646 peptide-binding site (Carroll et al. 2002) and odor formation based on soluble MHC
647 molecules (Pearse-Pratt et al. 1998; Janssen et al. 2001).

648 Although underrepresented, studies on MHC-odor interaction have also been
649 conducted on animals living in the wild or on wild-type animals held in captivity (n=
650 18 of 51 studies), and generally show support for a link between MHC and odor. For
651 example, in song sparrows (*Melospiza melodia*), black-legged kittiwakes (*Rissa*
652 *tridactyla*), and mandrills (*Mandrillus sphinx*) (Setchell et al. 2011; Leclaire et al.
653 2014; Slade et al. 2016; Grieves et al. 2019), there are positive correlations between
654 MHC genetic distance and chemical distance of the odor profile, the latter being
655 established using GC-MS. Of the two studies on captive ring-tailed lemurs (*Lemur*
656 *catta*), one found a statistically non-significant relationship between the absence of
657 certain MHC sequences and the concentration of volatile compounds in samples
658 obtained from the brachial gland and the tail (Knapp et al. 2006) while the other
659 found that MHC diversity and similarity is signaled via genital secretions in a sex- and
660 season-dependent manner (Grogan et al. 2019).

In addition to support from correlational studies, wild animals have been shown to discriminate MHC-based odor differences in conspecifics. For example, Arctic char (*Salvelinus alpinus*) discriminate between siblings who do and do not share the same MHC-genotype as themselves (Olsén et al. 1998). Similarly juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) spent more time in water conditioned by kin sharing MHC-alleles than in water conditioned by kin not sharing MHC-alleles when given the choice in a flow chamber (Rajakaruna et al. 2006). Captive ring-tailed lemurs also discriminate MHC-diversity in the genital odors of opposite-sex conspecifics as they spent more time investigating or reacting to genital secretions of MHC-similar compared to MHC-dissimilar scent donors (Grogan et al. 2019).

Despite the MHC's potential importance, external influences such as diet can have stronger impact on odortype (Brown et al. 1996; Kwak et al. 2008) and hinder discrimination of odortypes (Schellinck et al. 1993; Schellinck et al. 1997). Interestingly, odors lacking MHC-derived peptides have been discriminable (Singer et al. 1993) and carboxylic acids appear to play a role in shaping laboratory mouse odortypes and their discriminability (Singer et al. 1997). The circumstances under which the MHC is important in odor communication are therefore unclear and further research is warranted to detangle genetic from environmental influences on odor.

MHC and microbiota

Apart from directly influencing odor through shed MHC molecules or MHC peptide ligands, MHC-II has the potential to indirectly shape odor by governing microbiota (Fig. 2). In European plaice (*Pleuronectes platessa*), a weak but significant correlation between MHC-IIB matrices and pathogen abundance matrices of gill

686 microbiota was observed with certain alleles being positively linked to the presence
687 of certain bacterial genera (Wegner et al. 2012). In male Leach's storm petrels
688 (*Oceanodroma leucorhoa*) MHC-II DAB homozygosity explained 72% of variation in
689 the microbiota community structure of the uropygial gland (Pearce et al. 2017).
690 Similarly, Holstein dairy cows expressing two different MHC variants exhibit a
691 different composition of microbiota in their mammary glands on the day of calving but
692 not on following days (Derakhshani et al. 2018). These studies provide evidence for a
693 link between the MHC and the microbiota community, but they do not offer insights
694 into the mechanisms acting in MHC-based microbiota structuring.

695 Studies on blue petrels (*Halobaena caerulea*) (Leclaire et al. 2019) and sticklebacks
696 (*Gasterosteus aculeatus)* (Bolnick et al. 2014) present evidence for a negative
697 correlation between MHC diversity and microbial diversity (Table S3), supporting the
698 hypothesis that a diverse MHC genotype causes detection and elimination of more
699 microbiota species and thus a less diverse microbiota community. However, not all
700 studies found a negative relationship. For instance, in eastern hellbenders
701 (*Cryptobranchus alleganiensis bishopi*), individual MHC amino acid distance was
702 positively linked to microbial community richness (Hernández-Gómez et al. 2018).
703 Furthermore, in laboratory mice, MHC heterozygosity has been shown to enhance
704 functional diversity of the microbiome (Wadud Khan et al. 2019). The primary role of
705 the MHC-II in shaping the microbiota and its role in presenting extracellular rather
706 than intracellular peptides is also supported by Kubinak et al. (2015) who show that
707 MHC-II had a stronger influence on the microbiota than MHC-I.

708 Although our search strings did not yield publications linking all three components
709 (the MHC, microbiota, and odor), the search aimed at MHC-odor interactions yielded
710 a study investigating the influence of the MHC on both odor and the microbiota
711 (Zomer et al. 2009). It showed that in laboratory mice the MHC affected both volatile

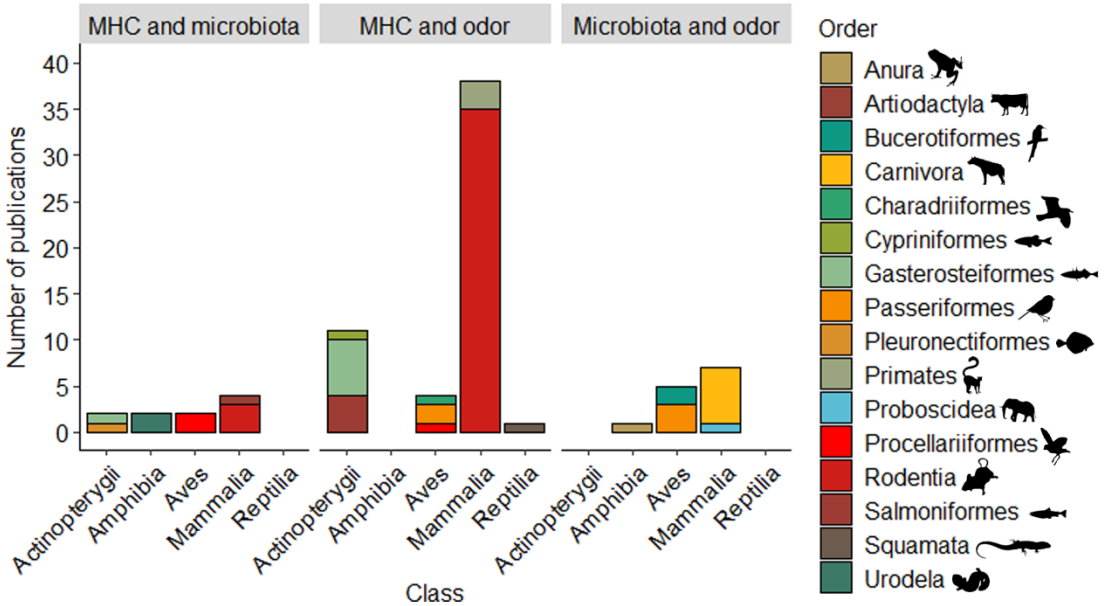
712 and microbiota profiles, however the effect of the MHC was weaker than the effect of
713 the genetic strain of the study animals. These findings are supported by another
714 study on laboratory mice indicating that both MHC haplotype and background
715 genotype impact odor profiles (Lanyon et al. 2007). However, although the study by
716 Zomer et al. (2009) included all three components, it did not investigate the link
717 between microbiota and odor, so it is unclear to what degree MHC-odor relationships
718 might be impacted by the microbiota. Furthermore, GC-MS was used to investigate
719 the effect of MHC on the odor profiles. While this is an appropriate technique for the
720 question in hand, it leaves unanswered whether animals can make use of these
721 subtle composition differences for social communication. Therefore, evidence of the
722 MHC and the microbiota acting on odor to provide reliable information for social
723 interactions has yet to be demonstrated.

724

725 **Composition of retrieved studies regarding study type and species**

726 Overall, results of our systematic review show that most studies focus on
727 correlational rather than causal investigation of interactions between MHC and
728 microbiota (n = 6 correlational vs n = 3 experimental studies). However, this pattern
729 is reversed for studies linking MHC and odor (n = 6 correlational vs n = 46
730 experimental studies; plus one observational/methodological publication), caused by
731 the great number of experimental studies on laboratory animals. For publications
732 investigating the relationship between microbiota and odor the proportion is almost
733 equal (n = 5 correlational vs n = 6 experimental studies). Altogether, publications
734 using laboratory-reared animals, mostly mice and rats, make up a similar portion
735 (37/73) compared to publications investigating wild or wild-type animals (36/73).

736 The phylogenetic composition of the study species used varies between the three
737 links investigated. Whereas rodents make up the majority of study animals for
738 publications investigating the link between MHC and odor (65%, 35/54, Fig. 4) with
739 the remaining portion of study species stemming from 8 different taxonomic orders,
740 study species of publications investigating MHC and microbiota and microbiota and
741 odor are more evenly distributed over five (microbiota and odor) and six (MHC and
742 microbiota) different taxonomic orders. The relationship between MHC and
743 microbiota and between MHC and odor has so far not been investigated in
744 carnivores, and for fish evidence for a link between microbiota and odor is missing.
745 Furthermore, there is a gap in publications investigating the link between MHC and
746 microbiota and microbiota and odor in reptiles and the interrelation between the MHC
747 and odor has not yet been investigated in amphibians.



748

749 Figure 4. Study species used in studies investigating the links between MHC and microbiota,
750 between MHC and odor, and between microbiota and odor. Number of publications that
751 investigated either the link between MHC and microbiota, the MHC and odor, and the

752 microbiota and odor is represented for the different classes. Within classes, publication
753 numbers are further broken down into taxonomic orders.

754

755 Compiling the empirical evidence for potential mechanisms regulating MHC-based
756 microbiota structuring showed that 5 publications retrieved in our systematic search
757 found a link between the composition of the MHC and the composition of the
758 microbiota community (Wegner et al. 2012; Kubinak et al. 2015; Pearce et al. 2017;
759 Derakhshani et al. 2018; Wadud Khan et al. 2019). In contrast, there were no
760 publications found that contest the link between MHC and microbiota composition
761 (Fig. 5), although publication bias of positive results cannot be ruled out. Publications
762 investigating the effect of MHC diversity on microbiota diversity also miss non-
763 significant results, showing support for two opposing hypotheses instead. Two
764 studies provide support for a limiting effect of MHC diversity on microbiota diversity,
765 causing a negative relationship (Bolnick et al. 2014; Leclaire et al. 2019) while
766 evidence for a positive relationship between MHC diversity and microbiota diversity
767 comes from a single study (Hernández-Gómez et al. 2018). Thus, further studies are
768 necessary to clarify whether the MHC has a role in affecting social odors through
769 shaping the microbiota community and to determine the potential mechanisms acting
770 between the MHC and the microbiota.

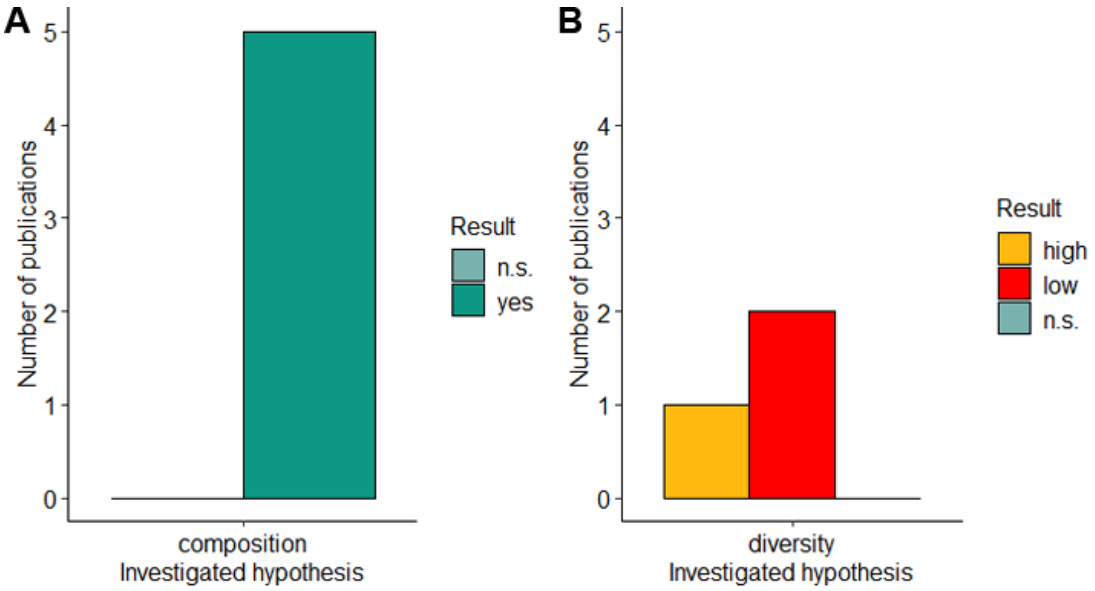


Figure 5. Empirical evidence for the relationship between MHC composition or diversity and the microbiota community. Number of publications investigating the link between MHC diversity or composition and the composition of the microbiota community (A) and MHC diversity or composition and microbiota diversity (B). Publications investigating the relationship between MHC composition or diversity and the composition of the microbial community (A) invariably provide evidence for a link between MHC diversity/composition and the composition of the microbial community ("yes") while no publications have been published that question this link due to non-significant results ("n.s."). Publications investigating the relationship between MHC diversity or composition and the diversity of the microbial community (B) either provide evidence for a negative correlation (high MHC diversity causing low microbiota diversity, "low") or for a positive relationship (high MHC diversity causing high microbiota diversity, "high"). There are no publications showing a non-significant relationship between MHC and microbiota diversity ("n.s.").

Knowledge gaps and future outlook

Despite 73 publications investigating the interaction of the microbiota and odor, the MHC and odor, or the MHC and microbiota, their results do not yield clear patterns

789 explaining the relations. Thus, we list several suggestions and recommendations for
790 future studies to develop credible evidence for the proposed mechanisms (Fig. 1 &
791 2).

792 (i) Findings on MHC-microbiota correlation are ambiguous and study numbers are
793 low. For wild mammals, evidence for any of the mechanisms governing these links
794 comes from a single publication only, which did not investigate the relationship
795 between MHC diversity and microbiota structure (Pearce et al. 2017). Our review of
796 the immunological processes points to possibilities for the MHC to both limit and
797 facilitate microbiota diversity (Fig. S5). Hence we argue researchers should
798 investigate whether patterns of MHC-microbiota diversity are consistent within
799 species with varying levels of MHC-II diversity. Studies involving a diverse range of
800 species and comparing the microbes of different body sites (including scent glands)
801 would be particularly beneficial as they will allow investigation of the circumstances
802 under which positive, negative and no relationships between MHC and microbial
803 diversity are found.

804 An alternative explanation of the mixed results between MHC and microbial diversity
805 is based on the optimality hypothesis (Nowak et al. 1992; Woelfing et al. 2009).
806 Imagine a U-shaped curve with microbial richness on the y-axis and MHC diversity
807 on the x-axis, where the optimum MHC allelic diversity has the lowest microbial
808 diversity. On the left side of the MHC optimum the relationship between MHC and
809 microbiota diversity would be negative. On the right of the optimum, the relationship
810 between MHC and microbiota diversity would be positive. Thus, to test the optimality
811 hypothesis multiple data points from the same study species at different MHC
812 variabilities (or different microbiota diversities) are required.

(ii) While there is clear evidence for the ability of wild animals to discriminate odor cues based on MHC in an experimental setting, there is a lack of studies demonstrating the application of this MHC-based discrimination of conspecifics for inbreeding avoidance or cooperation in order to increase fitness. We encourage studies on wild animals to verify use of this mechanism in a natural context. This could be performed in wild species for which the ability to discriminate has already been shown or on wild species for which, due to their behavior in mate choice or other social contexts, MHC-based odor discrimination may yield a substantial fitness benefit. MHC genotyping as well as odor and microbiota profiles combined with life history and behavioral data can provide evidence and thus help unravel whether decisions having severe fitness consequences are based on MHC-and microbiota-governed social odor cues in the natural context.

(iii) Researchers should base their experiments on sample sizes that allow reliable conclusions. The extreme polymorphism of the MHC makes it a promising target for governing odor cues used in social communication, but simultaneously it causes studies investigating the role of the MHC in shaping odor or the microbiota to require relatively large sample sizes in order to have enough power to detect small effect sizes (Gaigher et al. 2019). Researchers should consider the level of MHC polymorphism found in their study organisms and the likely effect size when designing their studies, for example by performing power analyses.

(iv) Researchers should be aware that both microbiota and odor are affected by genetic loci other than the MHC as well as exogenous factors. Studies have reported that other proteins, such as MUPs, play an important role in odor discrimination in mice (Cheetham et al. 2007) and that the mouse laboratory strain appears to have an even stronger impact on odor than the MHC (Zomer et al. 2009). However, MUPs are not universal to all species and we therefore recommend testing the influence of the

MHC while controlling for genetic similarity or relatedness (e.g. using high coverage SNPs, microsatellites or a pedigree) in order to disentangle the effect of the MHC from the influence of other loci.

(v) Our systematic review showed that studies focusing on MHC-microbiota and microbiota-odor interaction in wild animals mostly use correlational approaches and causal evidence is lacking. While experimental investigation of causal mechanisms is particularly difficult in wild animals, it is nonetheless necessary to demonstrate the usage of MHC- and microbiota-governed odor cues in social communication in a natural context. This could be achieved by artificially altering odor by adding MHC ligands (for example Milinski et al. 2005; Spehr et al. 2006; Hinz et al. 2013; Milinski et al. 2013) to the odor profile. Another option might be the modification of microbiota composition either with fecal transplants (reviewed in Lively et al. 2014) or with antibiotics (Gorman et al. 1974; Whittaker et al. 2019). However, antibiotic treatment might have additional confounding effects impacting odor. Furthermore, potential negative effects of antibiotics and the possibility of facilitating resistances in microbes should be considered when designing a study. Another functional approach is testing whether microbiota found in the commensal community of an animal produce odorants present in its volatile profile. Discrimination of odors produced by a host versus those produced by its microbiota is vital to uncover the microbiota's role in chemical communication.

(vi) Theories suggest that either MHC molecules themselves, the volatiles the MHC molecules might carry or volatiles developing due to the MHC's role in binding peptides could be potential sources of odor (Penn and Potts 1998a). However, what chemical components apart from MHC peptide ligands can enable or contribute to the discriminability of MHC-based odors has not yet been clearly determined. Most studies investigating MHC-governed odor profiles focus on GC-MS to determine the

volatile components of odor. Few studies have investigated the role of proteins in influencing odors governed by the MHC, with some showing that proteins or MHC molecules are not necessary for the discrimination of odor (Brown et al. 1987; Singer et al. 1993), that MHC molecules alone do not ensure odor discriminability, and that MHC cannot be discriminated through serum (Brown et al. 1987). Contrariwise, other studies investigating the role of proteins in the generation of odor show that injection of soluble MHC molecules or soluble MHC peptide ligands alters odor (Pearse-Pratt et al. 1998; Janssen et al. 2001; Milinski et al. 2010). These conflicting findings hint for a role of proteins such as MHC molecules themselves or their ligands influencing odor through binding or regulating volatiles rather than being an odor source themselves. Thus, we suggest that studies, apart from focusing solely on volatiles, should also look at other compounds such as proteins to help unravel the mechanism behind MHC-based odor regulation.

(vii) We need studies with a holistic approach combining interactions of all three components, the MHC, the microbiota, and odor, as, to our knowledge, no studies have investigated the links of all components simultaneously. For example, there is evidence that the MHC directly impacts on male Storm Petrels' microbiota composition (Pearce et al. 2017) and that odor profiles reflect genetic distance at the MHC (Leclaire et al. 2014; Slade et al. 2016; Grieves et al. 2019). However, causal links between all three are missing and it is unclear whether MHC, odor and microbiota are directly linked or if the MHC affects odor and the microbiota through separate mechanisms. Investigating the interconnections of all three in focal species could reveal the mechanisms underlying chemical communication and disclose the roles and interrelations of the MHC, the microbiota and odor.

Conclusion

The MHC-II as an essential part of the complex immunological network has the potential to affect the microbiota and consequently odor through various pathways. Findings regarding immunological mechanisms suggest that MHC-II diversity can potentially facilitate microbiota diversity by inducing tolerance rather than solely limit its diversity through elimination. However, the small number of empirical studies conducted thus far have produced mixed results, with some finding negative or no relationship. Insights from immunology provide great potential for unravelling MHC-microbiota-odor interactions by presenting new starting points and hypotheses, and we hope that this review stimulates advances in the investigation and understanding of this potential key pathway for social communication.

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Figure captions

Figure 1. MHC-microbiota interactions in chemical communication. Schematic of the interactions between genes of the MHC and the microbiota and their potential influence on odour. MHC polymorphism (blue arrows) might directly influence odour (solid arrows) through volatile and non-volatile by-products such as urinary signals or peptide ligands or indirectly (dashed arrows) by influencing infection status or through regulation of the microbiota (green arrow) producing volatiles.

Figure 2. MHC-microbiota interaction. (A) A negative correlation is characterized by high MHC diversity leading to low microbiota diversity. (B) A positive correlation may be caused by high MHC diversity tolerating more diverse microbiota communities. (C) Covariation between MHC genotypes and microbiota community structure may be caused by specific MHC binding motifs selecting for the presence of certain groups of microbes. (D) No detectable relationship between MHC and microbiota community may indicate the MHC is not a major determinant of the microbiota community.

Figure 3. Immune response. Steps of immune response involving MHC-II leading to (A) elimination and (B) tolerance of the pathogen. (A) (1) After recognition by an APC, the peptide is internalized, processed and (2) presented by the MHC-II. (3) Interaction of the MHC-II-peptide-complex with the TCR together with an inflammatory costimulatory signal cause Th cell activation. (4) Inflammation is further exacerbated through cytokine release by Th cells, (5) causing activation of cytotoxic T cells and increased proliferation of immune cells. Activated Th cells (6) activate B cells that (7) produce antibodies. (B) (1) The type of APC as well as (2) the

processing of the peptide can influence peptide recognition. (3) MHC-II and TCR strongly affect the set of presented peptides and the type of response. (4) MHC-II diversity is genetically determined, whereas the TCR repertoire is also determined by thymic selection. (5) ILCs can temper inflammation by inducing cell death of T cells acting against commensal bacteria. (6) In case of missing costimulation through an inflammatory signal, Th cell activation is prevented. (7) IgA produced by B cells can facilitate tolerance. (8) Treg cells promote IgA diversity and thus temper inflammation. Arrows displaying processes are colored in grey, cellular or humoral components are colored in green.

Figure 4. Study species used in studies investigating the links between MHC and microbiota, between MHC and odor, and between microbiota and odor. Number of publications that investigated either the link between MHC and microbiota, the MHC and odor, and the microbiota and odor is represented for the different classes. Within classes, publication numbers are further broken down into taxonomic orders.

Figure 5. Empirical evidence for the relationship between MHC composition or diversity and the microbiota community. Number of publications investigating the link between MHC diversity or composition and the composition of the microbiota community (A) and MHC diversity or composition and microbiota diversity (B). Publications investigating the relationship between MHC composition or diversity and the composition of the microbial community (A) invariably provide evidence for a link between MHC diversity/composition and the composition of the microbial community ("yes") while no publications have been published that question this link due to non-significant results ("n.s."). Publications investigating the relationship between MHC

diversity or composition and the diversity of the microbial community (B) either provide evidence for a negative correlation (high MHC diversity causing low microbiota diversity, “low”) or for a positive relationship (high MHC diversity causing high microbiota diversity, “high”). There are no publications showing a non-significant relationship between MHC and microbiota diversity (“n.s.”).

Figures

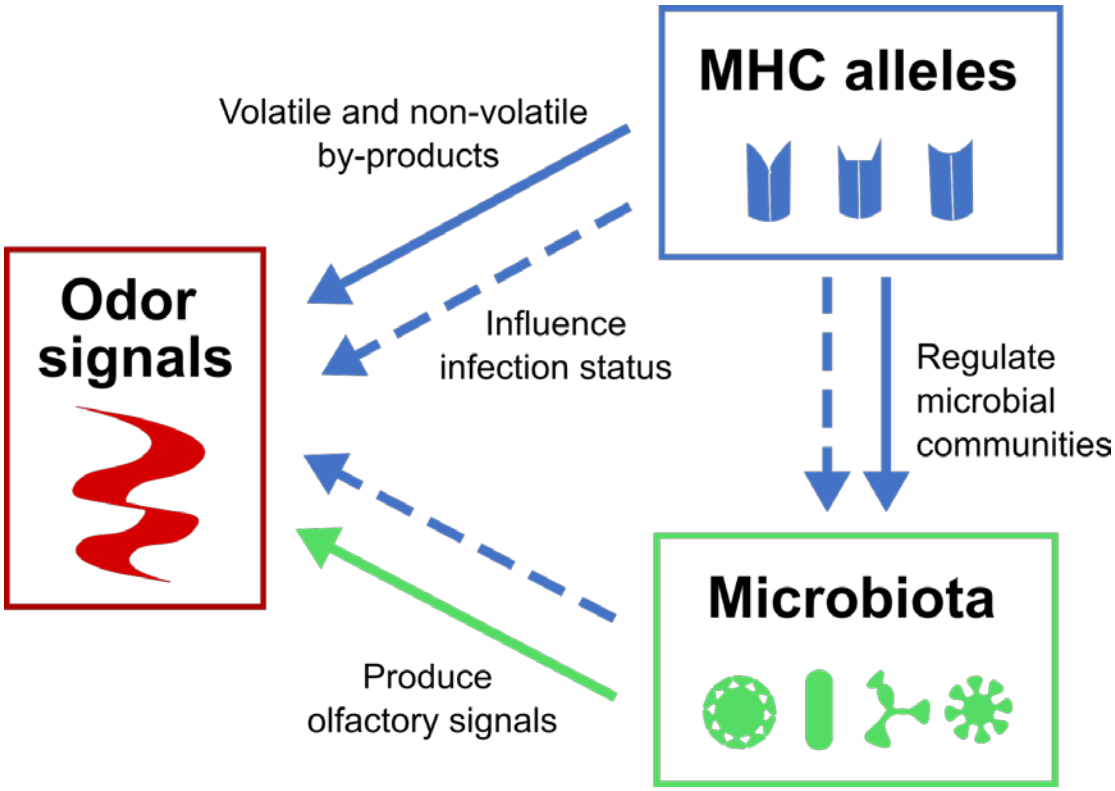


Figure 1

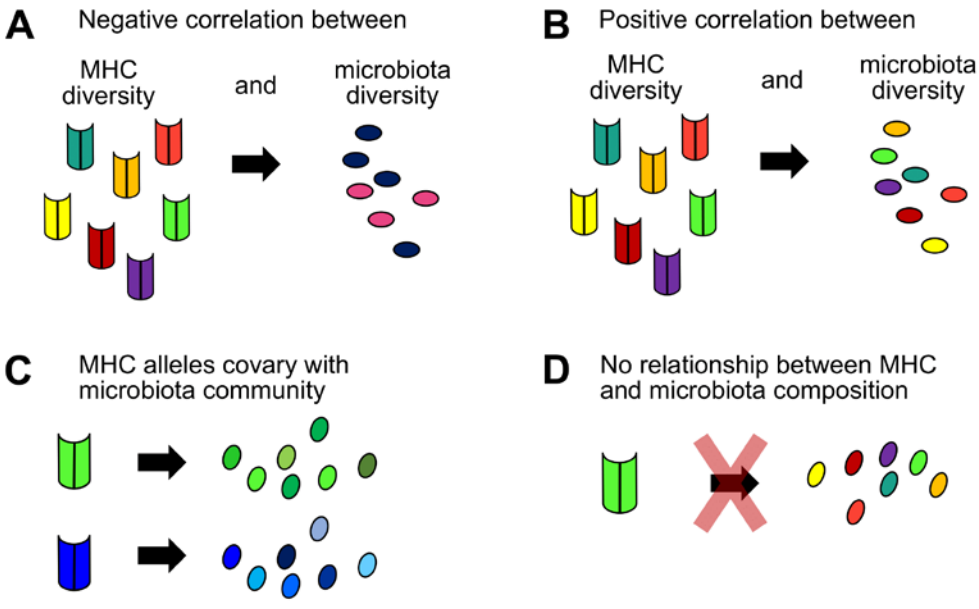
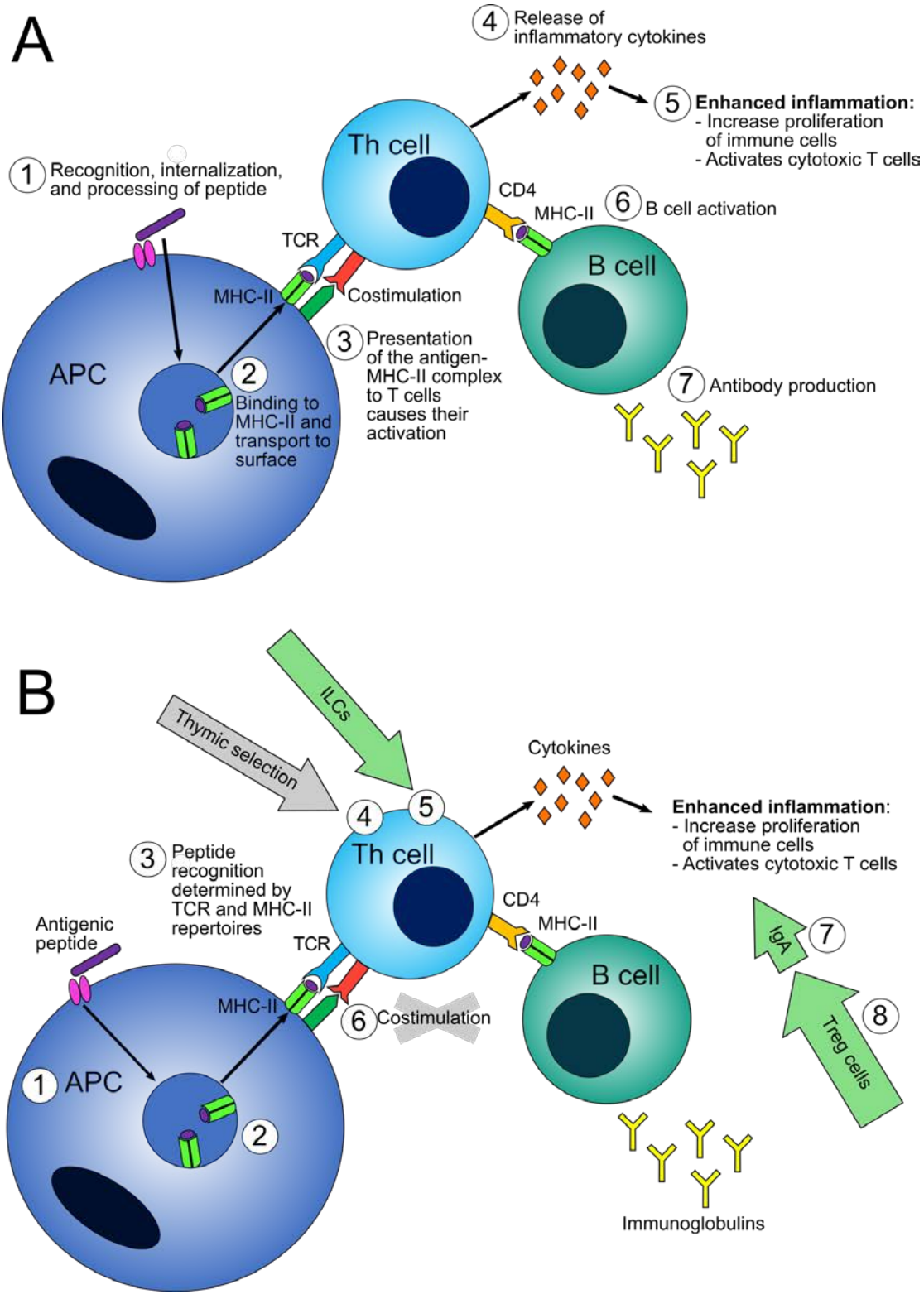


Figure 2



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1465 Figure 3

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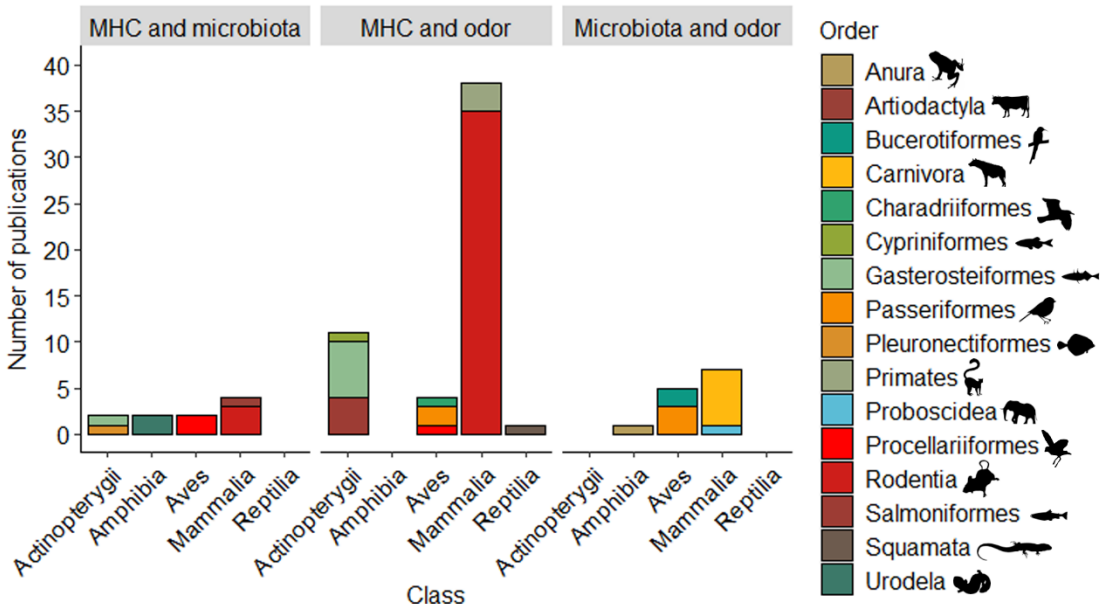

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1468 Figure 4

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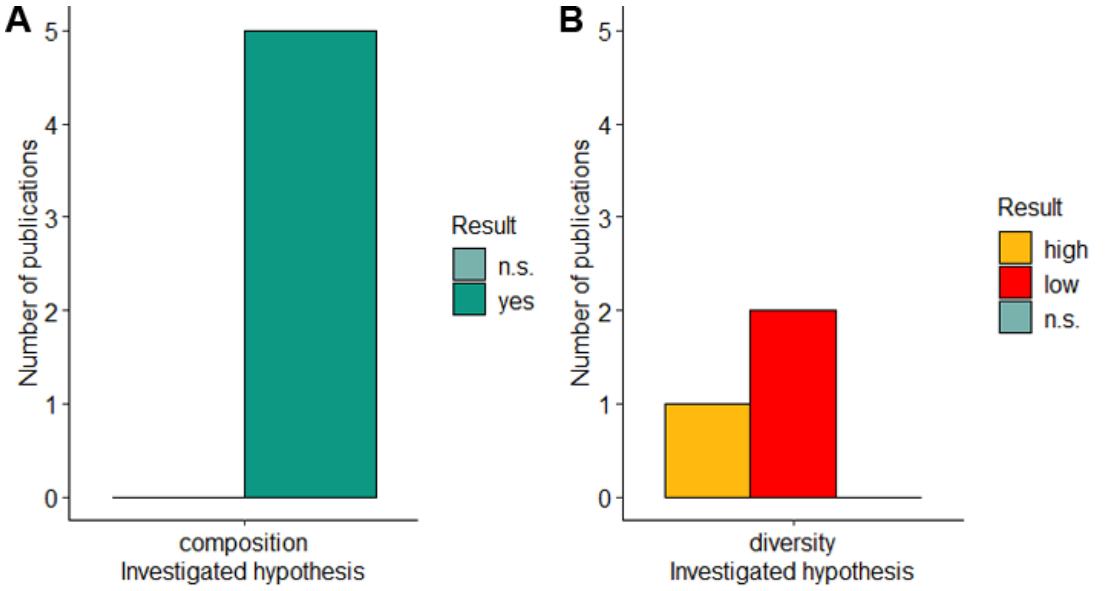

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1471 Figure 5