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Non-classical MHC class II positive cell types, function and immunological context

Neklasické MHC-II pozitívne typy buniek, funkcia a imunologický kontext

Bachelor's thesis

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V Prahe, 10.5.2019

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Abstract

Major histocompatibility complex class II (MHC-II) is a group of glycoproteins responsible for the presentation of exogenous antigens to T-lymphocytes. Besides the „classical“ antigen presenting cells (APCs), numerous cell types were proven to be able to express MHC-II molecules either constitutively or under specific conditions. Often, the stimulus for MHC-II expression is interferon γ, a pro-inflammatory cytokine typically activating promoter IV of the Class II Transactivator. Many of the non-classical MHC-II-expressing cells can serve as APCs, activating or attenuating T-cell proliferation depending on the expression of costimulatory molecules. Additional research identified some unusual functions of MHC-II molecules on non-classical cell types, including a role in prenatal development or mating. Modulation of the MHC-II expression could potentially serve many promising therapeutic purposes and new research can lead to deeper understanding of the topic.

Keywords: MHC-II, ILC, basophils, TEC, antigen presentation, CIITA, IFN-gamma

Abstrakt

Hlavný histokompatibilný komplex II. triedy (MHC-II) je skupinou glykoproteínov zodpovedných za prezentáciu exogénnych antigénov T-lymfocytom. Okrem „klasických“ antigén prezentujúcich buniek (APC) bolo potvrdených viacero typov buniek schopných exprimovať molekuly MHC-II buď konštitutívne, alebo za špecifických okolností. Častým stimulom expresie MHC-II je interferón γ, cytokín podporujúci zápal, typicky aktivujúci promótor IV transaktivátora II. triedy. Mnoho neklasických buniek exprimujúcich MHC-II môže fungovať ako APC, teda aktivovať alebo tlmiť proliferáciu T-lymfocytov, pričom záleží na expresii kostimulačných molekúl. Ďalší výskum identifikoval aj neobvyklé funkcie MHC-II molekúl na neklasických typoch buniek, vrátane úlohy v prenatálnom vývoji či párení. Ovplyvnenie expresie MHC-II by mohlo potenciálne slúžiť mnohým sľubným terapeutickým účelom a nový výskum môže viesť k hlbšiemu porozumeniu danej témy.

Kľúčové slová: MHC-II, ILC, bazofily, TEC, prezentácia antigénu, CIITA, IFN-gamma

List of abbreviations

AIDS	acquired immune deficiency syndrome
AH	autoimmune hepatitis
APC	antigen presenting cell
Are	aryl hydrocarbon receptor
BFU-E	burst forming unit erythrocyte
CD	cluster of differentiation
cDNA	complementary DNA
CFU	colony forming unit
CFU-GM	colony forming unit of granulocytes/monocytes
CIITA	class II transactivator
CIITA-p	class II transactivator promoter
CLIP	class II-associated invariant chain peptide
CLP	common lymphoid progenitor
cTEC	cortical thymic epithelial cell
DC	dendritic cell
EC	endothelial cell
ER	endoplasmic reticulum
FOXP3	forkhead box P3
GM-CSF	granulocyte-macrophage colony stimulating factor
HAT	histone acetyltransferase
HIV	human immunodeficiency virus
HLA-DR	human leukocyte antigen DR
IBD	inflammatory bowel disease
ICAM-1	intercellular adhesion molecule 1
IEC	intestinal epithelial cell
IFN-γ	interferon γ
Ii	invariant chain
IL	interleukin
ILC	innate lymphoid cell
IRF-1	interferon regulatory factor 1
ISC	intestinal stem cell
JAK	janus kinase
LAG-3	lymphocyte-activation gene 3
LFA-1	lymphocyte function-associated antigen 1
LNSC	lymph node stromal cell

MHC	major histocompatibility complex
MHC-II	major histocompatibility complex class II
NSC	neural stem cell
pIV	promoter IV
PKC	protein kinase C
STAT	signal transducer and activator of transcription protein
TAF	TBP (TATA-binding protein)-associated factor
Tat	trans-activator of transcription
TCR	T-cell receptor
TEC	thymic epithelial cell
Th	T-helper lymphocyte
TNF-α	tumor necrosis factor α
Treg	regulatory T-lymphocyte

Contents

1. Introduction

Antigen presentation is a process where naive T-cells become activated and proliferate into effector cells after recognizing an antigen. For exogenous antigens, the semi-digested peptide is presented by the major histocompatibility complex class II (MHC-II) molecule on the surface of an antigen presenting cells (APCs) to $CD4^+$ T-cells. These then serve as helper T-cells, producing cytokines and activating other cells in the immune system. T-cell deficiency or deficiency of MHC-II molecules have far reaching consequences and result in immune deficiency.

The role and the mechanism of antigen presentation and stimulation by the professional APCs is relatively well understood. However, evidence confirming involvement of several other cell types of the hematopoietic or non-hematopoietic origin has recently emerged. The accumulating evidence for the expression of *MHC-II* genes in various cell types points out that this phenomenon is probably more common than previously thought. The underlying mechanism for non-classical MHC-II expression is most often IFN-γ stimulation, although some exceptions have been found as well. There has been for long an uncertainty around the direct link between MHC-II expression and the function, including outcomes to the interaction with $CD4^+$ T-cells. Despite an extensive research, the functional significance and purpose of MHC-II molecules on some of the cells still remains unclear. Nonetheless, the relevant roles had been successfully determined for at least some cell types, sometimes with important functional consequences.

The aim of this thesis is to summarize current knowledge about the non-classical MHC-II expressing cells and to enlighten possible functions of MHC-II expression on these cells. It also hints possible directions of future research in this field of study.

2. MHC-II molecules

MHC-II is a group of heterodimeric glycoprotein molecules from the immunoglobulin superfamily traditionally viewed as expressed only on the surface of the „classical" APCs – macrophages, dendritic cells and B-lymphocytes. It consists of two structurally similar components, α and β chain, both of which contain two domains, a variable α_1 or β_1 domain, and a much more conserved α_2 or β_2 domain, with a transmembrane segment. The MHC-II is polygenic and polymorphic in enormous extent. The various genes for the MHC-II molecules evolved via gene duplications during last approximately 500 million years of the adaptive immunity history.

The synthesis of the MHC-II molecules begins in the rough endoplasmic reticulum (ER), where the α and β chains dimerize and form a terminal groove capable of binding short peptides with relatively low specificity, although having some preferred-residue pockets. To prevent binding and presentation of endogenous peptides in the ER lumen, a trimeric invariant chain (Ii) interacts with empty MHC-II dimer and physically blocks the peptide binding groove [1]. Nonameric (three αβ dimers trimerized by Ii trimer) complex travels from the ER through the Golgi complex up to the late lysosome (address is provided by the li) containing endocytosed digested molecules. Here, the Ii gets hydrolyzed except for a short peptide chain called the CLIP (class II-associated invariant chain peptide). Subsequently, the CLIP gets replaced by a foreign peptide with the help of a special type MHC-II molecule DM, which stabilizes the transitional state [2]. The MHC-II molecule with the bound peptide then moves to the cell surface.

2.1. Regulation of the *MHC-II* expression

Due to its specialized function, expression of the *MHC-II* has to be thoroughly regulated. Major regulator is the CIITA (Class II Transactivator) molecule controlling the expression of the *MHC-II* on a transcriptional level [3], although not directly interacting with DNA promoter. It cooperates with DNA binding factors, general transcription factors, and histone acetyltransferases (HATs) to initiate the transcription (reviewed by Ting and Trowsdale [4], see Figure 1). The control of the *MHC-II* gene transcription is maintained typically by interfering with CIITA transcription or function. An example of the latter is methylation of the transactivator by protein arginine methyltransferase 1 in macrophages. The methylated CIITA protein has a shorter half-life and is readily degraded. This could help to therapeutically down-regulate some chronical inflammation phenomena, such as atherosclerosis [5]. On the other hand, the MHC-II expression was successfully upregulated by viral vectors containing the gene for the master transactivator under the control of viral promoters, H6 and SP, the former being much more efficient. This could possibly lead to an increased immunogenicity of tumor or virus-infected cells [6].

On its own, CIITA (isoform III) have a relatively rapid turnover of less than 2 hours *in vivo*, thanks to its N-terminal proline/arginine-rich end. The latter is effectively preventing the stabilizing

modifications on the first amino acid, methionine, and thus making it prone to N-terminal ubiquitination, which subsequently leads to rapid degradation in the proteasome. However, the N-terminus also contributes to CIITA transactivating properties. Interestingly, monoubiquitination of several lysine residues further to the C-terminus of CIITA causes a prolonged half-life [7].

Three CIITA promoters have been identified. Promoters I and III are believed to be constitutive, promoter I is used mostly by dendritic cells and promoter III is typical for other hematopoietic cells (especially B-cells). Promoter IV has been found to be inducible by interferon γ (IFN-γ), which was first described as an underlying mechanism in MHC-II induction in macrophages and non-hematopoietic cells [8]. Nonetheless, it has been pinpointed that the promoter IV is transcriptionally active and functional in B-lymphocytes, as well as its induction by IFN-γ, which results in elevated levels of MHC-II compared to control. A possible therapeutic use of this fact has been proposed in targeting and eliminating particular hematological malignancies, multiple myeloma, which often escapes the immune system by downmodulated expression of MHC-II molecules. These cells usually have a functional promoter IV that could be activated to increase MHC-II expression under IFN-γ stimulation [9].

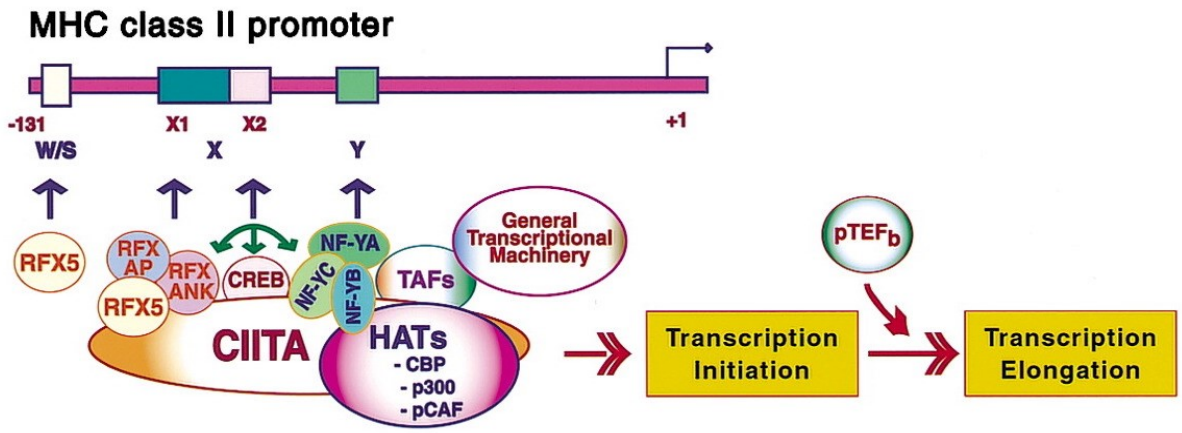

Figure 1: MHC class II transcription initiation. Note the role of all of the CIITA interactions across the trancriptional machinery. TAFs – TBP (TATA-binding protein)-associated factors, HATs - histone acetyltransferases. Arrows indicate interactions of the DNA binding factors with the MHC-II promoter. Figure reprinted from Ting and Trowsdale [4].

In non-hematopoietic cells, probably the most abundant way of inducing MHC class II expression is the IFN-γ, which acts as a transcriptional inducer [10]. The exact mechanism lies in a JAK1/2 (Janus Kinase 1 and 2) – STAT1 (Signal Transducer and Activator of Transcription protein) signaling pathway. STAT1 phosphorylation results in interferon regulatory factor 1 (IRF-1) expression, which acts as a transactivation element for *CIITA* transcription from promoter IV [3,11]. Promoter IV was found to be crucial for MHC-II expression in non-hematopoietic cells. In contrast, professional APCs, including macrophages, were fully functional in pIV$^-$ mice, other cell types lost their ability to upregulate MHC-II expression after IFN-γ stimulation. This may seem strange considering macrophages express

MHC-II in an activated state after IFN-γ signaling. To explain this phenomenon, existence of the additional putative promoter usage by macrophages was proposed. Consistently with this hypothesis, activated macrophages (including microglia) indeed express high amounts of *CIITA* type I mRNA and a little type III mRNA as well, on top of the previously discovered type IV. The other promoters may not be accessible in non-hematopoietic cells, resulting in different results compared to macrophages [12].

Further regulation of IFN-γ signaling pathway is complex. Several findings have demonstrated the significance of protein kinase C (PKC) for sufficient CIITA (and MHC-II) expression [13–15]. Different isozymes regulate the JAK-STAT pathway on various stages. For example, PKCε promotes phosphorylation of STAT1 by JAK in an integrin-dependent manner [16]. PKCα enhances the transactivation capacity of IRF-1 for CIITA expression, acting further downstream of the signaling cascade [17]. Furthermore, PKCδ is crucial for sufficient CIITA expression by additional STAT1 phosphorylation, causing increased HAT recruitment to the CIITA promoter. This mechanism is independent of the IRF-1 expression pathway and both processes are required for sufficient CIITA expression [18]. Taken together, PKCs, among other factors, are essential for CIITA expression induced by IFN-γ acting on several levels of cellular signaling.

Nonetheless, additional non-IFN-γ-dependent pathways of MHC-II expression induction have been found. To illustrate, a cell-contact dependent stimulation of endothelial cells (ECs) by natural killers (NK cells) is possible. For the activation, β_2 integrin/ICAM-1 (intercellular adhesion molecule 1) cell-cell interaction is needed. This pathway is more rapid than the classical IFN-γ pathway [19]. The reasoning behind is that it may not require additional protein synthesis, as it is also CIITA-independent[20]. A suspected mechanism could be protein tyrosine phosphorylation in response to ICAM-1 activation[21,22].

There may also exist other CIITA-dependent or independent pathways of MHC-II expression induction, however, they are currently poorly understood or may be restricted to special occasions or cells. Further research is required to fully embrace the whole problematics of MHC-II expression regulation.

2.2. Function of MHC-II

Regardless of the way of the expression inducement, the function of MHC-II lies mainly in the presentation of exogenous antigen peptides to naive $CD4^+$ T-lymphocytes. This function is generally accomplished by professional APCs discussed above. The process of activation and subsequent differentiation of the naive T-helper lymphocytes is called priming and takes place usually in the lymph node cortex. First, the T-cell and an APC adhere and form a dynamic immunological synapse. Second, if a T-cell receptor (TCR) interacts with the foreign peptide exposed on the MHC-II molecule, the adhesive interaction between the cells is stabilized due to the conformational change in LFA-1/ICAM-1 adhesive molecules driven by the cellular signaling in T-lymphocytes and their rapid movement

slightly slows down for a moment [23]. Afterwards, the T-cell undergoes several cell divisions, the daughter cells continue to bind to the local APCs. However, the recognition of an exogenous antigen by TCR signaling is not sufficient for the activation. T-cells have to receive also co-stimulation via CD4 co-receptor/MHC-II molecule interaction. The CD4 co-receptor adheres to the conserved part of the MHC-II molecule, namely a part of the β2 domain [24]. Other co-stimulatory molecules not interacting with the MHC-II/TCR are needed to promote T-cell survival and cytokines (such as interleukins IL-4, IL-6, IL-12 and IL-23) lead to the differentiation into a subset of T-helper cellular types. T-helpers consequently promote the immunological response in a specific manner according to the origin of the non-self antigen, humoral - antibody-mediated or a cellular inflammatory response.

The main function of MHC-II molecules expressed on classical APCs has been described here only generally. The main goal of the thesis is focused on non-classical MHC-II expression on hematopoietic and non-hematopoietic cells. Function of MHC-II molecules in this context is described in detail in chapter 5.

3. Non-classical expression of the MHC class II molecules on hematopoietic cells

When possible, the provided information is ordered universally. The expression in humans is generally followed by expression in mice, eventually, the *in vitro* results precede *in vivo* results.

3.1. Hematopoietic progenitor cells

Considering, that all of the professional APCs (except thymic epithelial cells) are derived from the hematopoietic stem cell, it is not surprising that some of the progenitors are MHC-II positive as well. However, it may be startling that the expression is not restricted to developmental lines pointing to the APCs.

For example, it is a well-known fact that pluripotent human progenitor cells, megakaryocyte progenitors and erythropoietic colonies all express HLA-DR molecules, a type of MHC-II in humans [25]. Recently, it was proven that megakaryocyte progenitors indeed interact with T-cells and can promote a Th17 or a Th17/Th1 response important for eliminating extracellular pathogens. However, mature megakaryocytes and platelets both already lack MHC-II [26].

In human myelopoiesis, the CFU-GM (colony forming units of granulocytes/monocytes) ratio to the BFU-E (burst forming unit erythrocyte) was decreased. It was mainly an effect of reducing numbers of granulocytes, monocytes being nearly unaffected, resulting in an increase of the monocyte/granulocyte ratio. This occurred as a shift in differentiation without the use of apoptosis [27]. Moreover, it has been shown that MHC-II expression on hematopoietic progenitors may also serve another function in cell suppression. The suppression impulse (e.g. in interleukin-3-induced CFUs) comes from regulatory T-cells (Tregs) producing tumor growth factor-β [28].

Moreover, the expression of MHC-II changes over the course of ontogenesis. In mice, MHC-II molecules are absent on the cell surface from early B-cell progenitors (pro-B) through pre-B-cells to immature B-lymphocytes in fetuses, whereas the MHC-II molecules are present from pre-B-cells to later stages in adults [29].

As the evidence suggests, it seems that the expression of MHC-II on hematopoietic progenitors is tightly regulated and serves many distinct and often unique functions. It is also clear that the level of MHC-II expression varies in the course of differentiation.

3.2. T-cells

It has been known for decades that activated T-lymphocytes of various species, including humans, express MHC-II [30]. The expression is regulated via CIITA promoter III (CIITA-pIII) ensuring the CIITA production [31].

The MHC-II expression on $CD4^+$ $CD25^{high}$ T-cells also helped to define a new functional subpopulation among human Tregs. The MHC-II^+ Treg cells participate in the early contact-dependent

suppression of $CD4^+$ effector T-cell proliferation and in production of IL-4 and IL-10 cytokines. On the contrary, the MHC-II^- subpopulation first enhances IL-4 and IL-10 production and mediates contact-dependent suppression subsequently, a few days later. The expression of FOXP3 (forkhead box P3) protein, a master regulator of the development and function of Tregs, was linked to the contact-dependent suppression in both cases. However, the MHC-II^+ subtype was found to contain higher levels of FOXP3. Interestingly, the initial observation of MHC-II expression was not linked to the long-term MHC-II expression, rather, the $CD25^{high}$ was. These findings imply that the $CD4^+$ $CD25^{high}$ MHC-II^+ population is a functional subset of mature suppressive Tregs rather than a separate lineage of T-cells[32].

However, despite numbers of experiments, the expression of the MHC-II as well as the expression of CIITA has always failed to be proven in murine T-cells. Nonetheless, an insertion of a functional copy of the human CIITA cDNA into murine lymphocytes resulted in the induction of MHC-II expression [33]. As an explanation, methylation of the CIITA-pIII has been proposed, a similar phenomenon described in T-cell acute lymphoblastic leukemia [34]. However, this does not imply that the murine T-lymphocytes cannot gain MHC-II molecules at the cell surface. In fact, an acquisition of MHC-II complex from the professional APCs was observed, along with co-stimulatory molecules, such as CD80 via trogocytosis. This includes switching of membrane patches between cells. The acquisition made it possible for murine T-cells to present antigens to other T-cells just as in other species, with the results varying, depending on a state of a recipient cell. If the recipient cell was in a resting state (being negative for MHC-II), the interaction resulted in proliferation. However, if the recipient cell was already activated and the antigen presentation and recognition was both already happening, the outcome of additional MHC-II recognition was either anergy or apoptosis in various ratios. The proposed hypothesis is that this type of behavior could lead to the reduction of clonal expansion of T-cells [35].

Nevertheless, humans are not the only species to express MHC-II molecules on their T-lymphocytes. Rather, it is a property spread widely among the animal kingdom. Several decades ago, the expression was confirmed on equine T-lymphocytes [36]. Both $CD4^+$ and $CD8^+$ T-cells were found to express MHC-II molecules. Intriguingly, the expression was found to be much higher in adult horses compared to neonatal foals. A suggested explanation for this fact was the formation of memory T-lymphocytes expressing MHC-II molecules on their surface [37]. Additionally, a fluctuation in the level of expression was linked to the MHC haplotype. In particular, haplotype D3 showed to decrease the expression significantly among all lymphocytes, but the reason behind the observations is yet to be explained [38]. Since these observations, no new results in the problematics have been published.

Another species with an experimentally proven T-lymphocyte MHC-II expression is a swine. Four subgroups of resting, extrathymic T-lymphocytes, differing in the expression of CD4 and CD8 co-receptors, were identified. These involve combinations exclusive to swine, e.g. $CD4^+$ $CD8^+$ and $CD4^-$ $CD8^-$ T-cells. Simultaneously, a constitutive expression of MHC-II was revealed on $CD4^-$ $CD8^+$ and $CD4^+$ $CD8^+$ resting populations. In contrast, none of the $CD4^-$ $CD8^+$ and few of the $CD4^+$ $CD8^+$ thymocytes have been found to express MHC-II molecules [39]. Subsequently, a function of the MHC-II

expression in swine T-lymphocytes was confirmed. Both subsets of $CD8^+$ T-lymphocytes can act as APCs, even when naive and inactive [40]. Upon exposing purified $CD4^+$ $CD8^+$ and $CD4^+$ $CD8^-$ subpopulations to viral antigens, only the former population got activated and proliferated. A MHC-II-restricted helper T-lymphocyte reaction was identified, with $CD4^+$ acting as a restriction element. This subset later evolved into antigen-specific memory Th lymphocytes [41].

It has been demonstrated that T-lymphocytes of many species often express MHC-II molecules on their surface. However, there are many variations in timing and other specificities among the species. MHC-II molecules on T-lymphocytes can serve multiple functions and are often expressed under specific circumstances or after exact stimuli.

3.3. Innate lymphoid cells (ILCs)

Innate lymphoid cells are a group of lymphocytes originating from the common lymphoid progenitor (CLP). In contrast to B- and T-cells, they lack antigen-specific receptors. They are also non-cytotoxic, therefore distinct from NK cells (although some authors group NK cells along with ILC1). They are thought as innate counterparts of Th cells, and are further subdivided into three subcategories: ILC1, ILC2 and ILC3, promoting the Th1, Th2 and Th17 immune responses, respectively. Upon activation, which takes place through cytokines or microbial products, they produce effector cytokines identical to those produced by Th cells of given categories (see Figure 2). The effector cytokines promote both innate and adaptive immunity responses, promoting specific differentiation of $CD4^+$ T-lymphocytes into their respective subsets (reviewed by Eberl *et al.* [42]). Moreover, it has been demonstrated that Th2 $CD4^+$ T-lymphocytes are further capable of mutual ILC2 stimulation by IL-2 cytokine production [43]. Thus, they contribute dramatically to the function of immune system by connecting both innate and adaptive components.

Furthermore, ILCs can also directly influence $CD4^+$ T-lymphocytes by cellular contact thanks to their MHC-II expression. These MHC-II-positive cells were first described according to their expression pattern, however, they were named and accepted as a special cellular type later (now under ILC3) [44]. ILC2 reportedly enhance T-cell proliferation and Th2 development by antigen presentation on MHC-II. However, MHC-II may not be crucial to promote Th2 response under all circumstances. In the company of other sufficient TCR stimuli, another further unexplored cell contact-dependent MHC-II-independent mechanism is enough to drive Th2 differentiation [43]. On the other hand, a subset of ILC3 inhibits $CD4^+$ cell responses by using MHC-II molecules. It is due to the innate cells lacking other costimulatory molecules such as CD80 and CD86 on their surface, thus resulting in anergy rather than stimulation of $CD4^+$ T-lymphocytes reactive to the presented antigen. This has numerous consequences in preventing chronic gut inflammatory diseases, such as inflammatory bowel disease (IBD), as a result of hindering pathologic T-cell responses to commensal gut bacteria. Those responses are in the absence of MHC-II expression prevented only in germ-free mice or by a continuous usage of antibiotics [45].

Further research has led to a conclusion that MHC-II expression on ILC3 is under the control of CIITA-pIV promoter. Dysregulation of MHC-II expression is present in patients with IBDs such as Crohn's disease, leading to elevations in the levels of Th17 cells and commensal bacteria-specific immunoglobulin G. Moreover, it has been proven that ILC3 act also as withdrawing agents of IL-2 from $CD4^+$ T-lymphocytes thanks to their higher binding capacity. This fact, together with the lacking costimulation upon antigen presentation on MHC-II molecules, can lead not only to anergy, but also to T-cell apoptosis, reducing the numbers of commensal bacteria-reactive T-helpers [46]. Taken together, all these processes are unbalanced in IBD patients, making them prone to idiopatic chronic inflammation of gut.

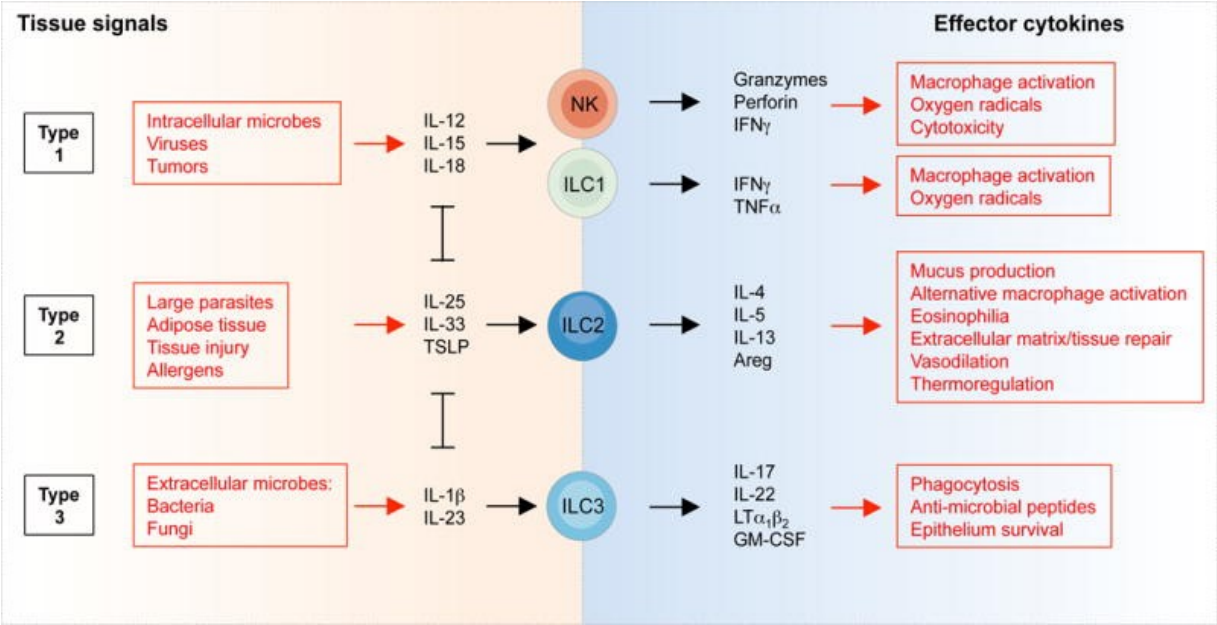

Figure 2: The function of ILCs. The stimuli in red brackets on the left affect the ILCs by respective stimulatory signal cytokines they induce. The ILCs activate upon stimulation and produce effector cytokines with various effects (red brackets on the right). Figure reprinted from Eberl et al. [42].

ILCs were observed not only in inflammatory environment, but also in tumors. ILC1 infiltrate malignant gastrointestinal tumors (although the expression of MHC-II in such location was not found). Furthermore, considerably higher amounts of ILC2 were detected in malignant breast cancer tissue, along with an increase in expression of MHC-II. However, it remains unclear how these findings could be used therapeutically in the future [47].

In summary, ILCs are an extremely important group of cells with numerous functions interconnecting innate and adaptive immunity or other. Upon stimulation, they can act either by producing effector cytokines, in a MHC-II-dependent manner or other contact-dependent stimuli, mostly interacting with $CD4^+$ cells. Understanding their function and finding new therapeutic approaches to affect their responses may be crucial in the future for fighting chronic inflammation disorders or cancer, among other things.

3.4. Basophils

In terms of MHC-II expression, basophils are perhaps one of the most controversial cell types. Several years ago, three independent studies confirmed that basophils indeed express MHC-II molecules in mice as well as costimulatory molecules CD80 and CD86, making them potent APCs [48–50]. The expression was suggested to be endogenous because of CIITA production [50]. Additionally, basophils seem to be necessary and sufficient for the induction of the Th2-type cell response mediated by IL-4 production. As an important specific cytokine attracting them to periphery was shown to be thymic stromal lymphopoietin, which they also produce once activated. After exposure to allergens, such as papain, they move from bloodstream to draining lymph nodes where they produce cytokines generating Th2 response [51].

However, subsequent findings questioned the proposed role of circulating basophils as APCs in humans as well as their capability to express significant levels of MHC-II. A recent study failed to demonstrate MHC-II expression on circulating basophils even after exposing them to several allergen types, nor was it able to note any Th2 polarization attributed to basophils. The study pointed out that previous experiments were based almost solely on basophils extracted from lymph nodes or spleen and that circulating basophils cannot be viewed on as APCs [52]. Nevertheless, it was shown that after using a broader-spectrum antibody against three human MHC-II subtypes, or just different antibody against the „main“ subtype HLA-DR, a small portion of basophils could be labeled as MHC-II positive. The results also indicated that the portion is similar in both sensitized and non-sensitized donors. However, the functionality of the MHC-II-dependent stimulation was not determined [53]. Later, it was uncovered that basophils indeed could, in up to 17% cases after 3 days, express MHC-II molecules at both RNA and protein level. This was achieved by stimulation of basophils by cytokines IL-3, IFN-γ and GM-CSF (granulocyte-macrophage colony stimulating factor). However, it was pinpointed that the expression of costimulatory molecules had been very low and activated basophils had been unable to promote Th2 differentiation [54].

A likely conclusion to the controversy was provided recently. Basophils were found to acquire MHC-II molecules from dendritic cells (DCs) via a process called trogocytosis, just as in the case of murine T-cells gaining MHC-II from APCs. These findings could help to explain contradictory evidence from various experiments depending on the methods used, as basophils could earn MHC-II molecules prior to the measurements from DCs in the bone marrow. After acquiring MHC-II molecules (together with costimulatory molecules), basophils could indeed function as APCs and drive Th2 differentiation[55]. However, further supporting evidence is needed to clarify the results and unite all controversial theories.

3.5. Eosinophils

It is worth notice that mature human eosinophils from blood can also synthetize and express MHC-II molecules on the surface under the influence of GM-CSF, IL-3 and IFN-γ *in vitro*, while also expressing

adhesive molecules like ICAM-1 [56,57]. After stimulation, they express costimulatory molecules, CD40, CD80 and CD86, as well, and are therefore able to stimulate T-cell proliferation upon induction, possibly with the help of ICAM-1 molecules required to form immunological synapse [58–60].

Finally, eosinophils from sputum of asthmatics, though not from blood, were detected to express MHC-II molecules *in vivo*, along with ICAM-1 [57]. It is therefore possible that eosinophils do not physiologically serve as APCs, though in the pro-inflammatory environment, they serve an important role in maintaining the inflammatory response by antigen presentation, among their other functions.

3.6. Neutrophils

Following basophils and eosinophils, it was established that human neutrophils can also be stimulated *in vitro* to express MHC-II using GM-CSF, IL-3 and IFN-γ. Although generally short-lived, upon stimulation were neutrophils able to prolong the lifespan [61]. It was also shown that human neutrophils can serve as functional APCs, but not as effective as DCs. The results hinted that for the MHC-II expression *in vivo*, the antigen was not a sufficient stimulation. Rather, the presence of antigen-specific $CD4^+$ T-lymphocyte was also needed. Lower levels of MHC-II molecules on the surface under physiological conditions suggested, that in humans, neutrophils may not be adequate for priming of naive T-lymphocytes and only serve to activate memory T-cells with a lower threshold required for activation. It was also proposed that in some cases, neutrophils may actually lower antigen presentation by DCs by their ability to uptake and dispose antigens, making them ineffective as APCs as well [62].

However, different experiments illustrated that murine neutrophils could actually express costimulatory molecules CD80 and CD86 and prime T-cells into Th1 and Th17 responses [63]. To quantify neutrophil antigen-presenting abilities and outcomes, further research using DC-deficient models is needed.

4. Expression of the MHC class II molecules on non-hematopoietic cells

4.1. Thyroid cells

Thyroid cells have for long been known to express MHC-II antigens under autoimmune circumstances. Presence of the MHC-II molecules was proposed to be an early marker of various pathological conditions in the thyroid gland in humans, such as autoimmune lymphocytic thyroiditis [64]. The level of lymphocytic infiltration of the inflamed thyroid gland seems to correspond to MHC-II expression levels. The underlying mechanism could be clarified by IFN-γ-dependent activation, since *in vitro* stimulation of thyroid cells by either IFN-γ or lectin resulted in an elevated MHC-II expression[65].

Likewise, thyrotropin hormone was also found to be a stimulatory factor in combination with IFN-γ in rats [66]. It has been therefore proven that under pro-inflammatory stimuli, thyroid cells express MHC-II. However, the evidence for the observations comes from fairly old publications, while newer ones focused mainly on other raising questions, such as possible treatment options. Those are not being specifically discussed further.

4.2. Pancreatic islet cells

Autoimmune pancreatic disorders, such as diabetes type 1, are triggered by the inflammatory detrimental process resulting in destruction of pancreatic islet cells (namely beta cells producing insulin). In the pathogenesis of the disease, a link to the MHC-II expression was found in mice. Under IFN-γ stimulation, beta cells were able to express MHC-II molecules in medium levels, but sufficient for the activation of memory $CD4^+$ T-lymphocytes. However, little or no expression of costimulatory molecules (CD80 and CD86) was found, possibly resulting in anergy of interacting T-cells [67]. In contrast, islet endothelial cells could express MHC-II after IFN-γ induction along with costimulatory molecules, possibly giving them the ability to stimulate $CD4^+$ T-cells. Nonetheless, they seem not to be crucial for the onset of diabetes or insulitis. However, considering the timing of MHC-II expression in endothelial cells much prior to beta cells MHC-II expression and therefore any other detectable sign of inflammation, it could be an early diagnostic indicator of insulitis [68].

4.3. Hepatocytes

MHC-II molecules were observed on the hepatocyte cell surface after IFN-γ stimulation long ago, both in viral hepatitis [69] and other chronic liver diseases in humans [70]. Autoimmune hepatitis (AH) has been linked to specific MHC-II alleles in both humans and dogs. As for humans, several allele variants represented different susceptibility and severity of the disease [71]. Clarifying the pathophysiology of AH, anti-MHC-II antibodies were found in the serum of patients with both classic AH and AH following

liver transplantation, corresponding to liver damage [72]. In dogs, alleles both lowering and increasing the probability for disease development have been found [73].

Hepatocytes in mice were also endowed with costimulatory molecule expression, enabling them the function of stimulatory APCs for $CD4^+$ T-cells. However, despite beliefs, the experiments implied that even MHC-II overexpression was not sufficient to initiate autoimmune disease [74].

In viral hepatitis, MHC-II molecules are tightly linked to mechanisms leading to chronicity of the disease. The Hepatitis C Virus blocks the antigen presentation by MHC-II molecules in both hepatocytes and DCs in chronically infected patients. The underlying mechanism could be explained by the reduced production of Cathepsin S, which normally cleaves the invariant chain. This leads to decreased immunogenicity and presentation of viral antigens and consequent weak immune response, resulting in the inability to eliminate the pathogen [75].

Additionally, antigen presentation and priming of T-cells by hepatocytes leads to differentiation into Th2 cells while also irreversibly attenuating IFN-γ production by Th1 lymphocytes in mice. This leads to decreased the inflammatory response, resulting in prolonged or chronic disease [76].

4.4. Epithelial cells in the gut

MHC-II molecules were detected in humans on a variety of epithelial cells, including the oral cavity (tongue and tonsils), urethra, epididymis, proximal renal tubules and several parts of the respiratory and gastrointestinal tract [77]. The latter two will be described further.

MHC-II is localized on small intestinal epithelial cells (IECs) in a defined patchy pattern in the apical part of the columnar cells. The magnitude increases from the base to the tip of the villi [78]. This stratification is also visible during the human fetal development, with expression starting from villus tips from 18th week of gestation [79]. On the epithelial cells covering Peyer's patches, only M cells showed reproducible expression of MHC-II, other follicle-associated epithelial cells had only minor detectable expression [80]. Regarding physiological processes, only the antigens originating from the apical surface of the IECs could be processed and presented (on the basolateral surface) on the MHC-II molecules. Antigens internalized from the basolateral surface could initiate MHC-II processing only after CIITA overexpression [81].

While constitutively expressing MHC-II molecules, it was unclear whether IECs also express costimulatory molecules needed for T-cell activation and proliferation. It was shown that even though mRNA molecules for CD40, CD80 and CD86 were detectable, no physiological surface expression was observed on duodenal cells, indicating the possibility of promoting anergy instead of T-cell activation[82]. However, inflammation can stimulate in an IFN-γ-dependent manner expression of CD86 in IECs [83]. CD40 expression was also found on the basolateral surface of IECs in patients with active IBD [84]. This fact may help to explain the pro-inflammatory properties of intestinal mucosa in patients with IBD compared to the physiological anti-inflammatory milieu in healthy individuals.

Interestingly, IFN-γ stimulation had no effect on costimulatory molecule expression in healthy mucosa in mice [85]. Therefore, more pro-inflammatory cytokines may be needed to induce costimulatory molecule expression in mice.

IECs could also affect T-cell proliferation indirectly via the secretion of exosomes, vesicles 30-90 nm in diameter containing MHC-II, from both apical and basolateral surface in humans [86]. Subsequently, released exosomes bind to DCs and enhance T-cell priming [87]. From the observations in mice, exosomes, being modulated by IFN-γ, were first considered as pro-inflammatory structures [88]. However, a type of exosomes named „tolerosomes“ had been found in rats, produced by IECs and bearing MHC-II. These structures acted in the anti-inflammatory manner and were responsible for antigen-specific tolerance in naive test subjects [89]. Further investigation is needed to fully understand the importance of exosomes in protective as well as inflammatory responses.

To conclude, MHC-II molecules are constitutively expressed on IECs and are capable of antigen presentation, resulting in both tolerogenic and pro-inflammatory responses depending on the circumstances. Exosomes produced by IECs stand for the indirect pathway influencing immune responses in the gut. Other roles of MHC-II expressed by IECs and some frequent pathologies originating from their imbalance are being discussed further in chapter 5.3.

4.5. Epithelial cells in the respiratory system

In contrast with the undoubted constitutive MHC-II expression within small intestinal epithelium, the expression of MHC-II molecules in respiratory tract remains a highly discussed topic.

At first, a strong, ubiquitous expression was found in humans on the epithelial surface from major bronchi further downstream up to the bronchioles and alveoles [90]. Further evidence confirmed the expression of MHC-II on human ciliated bronchial epithelial cells, along with mRNA transcripts. However, the expression decreased over the course of 5 days in culture, although under the stimulation by IFN-γ, the expression remained stable. Nonetheless, the ciliated bronchial epithelial cells failed to stimulate T-lymphocytes without proper stimulation with exogenous substances [91], which may lead to a conclusion that ciliated bronchial epithelial cells are normally incapable of proper T-cell stimulation, even when expressing MHC-II molecules. As proposed, the elevated levels of MHC-II were also observed in asthmatic patients and in chronic bronchitis [92], which is another example of MHC-II expression in inflammatory environment.

However, other studies working on rat bronchial epithelial cells raised doubts on the permanency of MHC-II expression in this model, when the expression and the capability to activate T-cells was undetectable unless treated with IFN-γ [93]. As for bronchial epithelial cells, it may be therefore possible that humans and rats differ in some physiological aspects regarding MHC-II expression, or that the detection systems were not equally sensitive.

Moving on from bronchial epithelial cells, type II pneumocytes showed constitutive expression in humans as well [94], but T-cell stimulation was proven to be ineffective, partially because of the lack of costimulatory molecules [95]. IFN-γ treatment was able to enhance MHC-II density as well as the capability to activate T-cells [96]. T-cell activation could be related to the expression of costimulatory molecules in both bronchiolar and alveolar epithelia, occurring *in vivo* in various pathological conditions, such as idiopathic pulmonary fibrosis and bronchiolitis obliterans-organizing pneumonia [97]. Inhibition of costimulatory molecule expression could also help to prevent lung transplant rejections, where CD80 and CD86 were detectable in humans within 3 months after transplantation [98]. Deeper knowledge of the MHC-II expression in respiratory tract could help fighting various respiratory disorders, including chronical ones, and weaken the effects of many prevalent diseases such as asthma.

4.6. Intestinal stem cells

MHC-II molecules on murine intestinal stem cells (ISCs) were identified only recently. ISCs were proven to be functional as a non-classical APCs and the MHC-II levels were elevated during infections, possibly as the effect of IFN-γ-producing Th1 lymphocytes. While the exact role of MHC-II molecules on ISCs remains unknown, the role in ISC renewal and differentiation has been proposed. On one hand, Tregs were shown to induce ISC self-renewal and increase ISC pool after inflammation. On the other hand, Th1, Th2 and Th17 all promoted ISC cell differentiation in a pro-inflammatory state, favoring Paneth (Th1) or tuft (Th2) cell differentiation. Th17 seemed to repress self-renewal without shifting the ratio of differentiated cells [99].

In summary, MHC-II expression could play an important role in maintaining the ISC pool and in both pro- and anti-inflammatory responses in the small intestine, while also functioning as non-professional APCs [99].

4.7. Subepithelial myofibroblasts

Subepithelial myofibroblasts, or stromal cells, were found to express both MHC-II molecules and costimulatory molecules CD80 and CD86 in humans. The expression is continuous and sufficient for allogenic $CD4^+$ T-cell priming. The localization of subepithelial myofibroblasts enables them to process antigens that crossed colonic epithelial barrier. The antigens can be directly presented to the residing lamina propria $CD4^+$ T-lymphocytes, resulting in a nearly instant reaction to foreign antigens [100].

4.8. Thymic epithelial cells

For the intact T-cell maturation and immune system function, both positive and negative selection has to take place in thymus. The former takes place in the cortex, the latter in the medulla [101]. The MHC-II molecules in the cortex can be found on the surface of epithelial cells and the expression is crucial for positive selection of T-cells in both mice and humans [102,103]. Taken from a different point of view, MHC-

II expression on cortical thymic epithelial cells (cTECs) alone was sufficient for positive selection in mice, even when all other cells, including professional APCs, lacked any functional CIITA promoter[104].

Interestingly, it was found that cTECs use the pIV CIITA promoter, just as other non-hematopoietic cells, although their CIITA and MHC-II expression appears to be constitutive *in vivo* [12]. PIV^- mice, without non-hematopoietic MHC-II expression, exhibited drastic reduction in numbers of $CD4^+$ T-cells, while the level of $CD8^+$ and double-positive lymphocytes did not decrease and negative selection was not abrogated. Additionally, medullary TECs were observed to be MHC-II positive as well, losing the positivity in pIV^- mice [105].

Hence, it is not clear whether cortical, medullary, or both groups of TECs are responsible for transfer of MHC-II molecules to T-lymphocytes, a process abolished by pIV deletion. In experiments with IFN-γ, IL-7 and various other substances, none were defined as crucial for MHC-II expression on their own. It is therefore possible that many stimuli collaborate into the final, MHC-II positive phenotype, and that they are partially redundant [105].

4.9. Lymph node stromal cells

Lymph node stromal cells (LNSCs, consisting of fibroblastic reticular cells, lymphatic endothelial cells, blood endothelial cells and a „double negative“ cells) use pIV promoter to express endogenous MHC-II molecules. However, they can also gain a significant amount of MHC-II molecules from DCs via exosomes, trogocytosis or potentially by nanotubes. Remarkably, their effects on naive $CD4^+$ cells in mice are anti-proliferative, pro-apoptotic or tolerogenic [106]. In pIV^- mice, the lack of or lowered MHC-II expression resulted in autoimmunity, with elevated effector T-lymphocyte levels [107]. In spite of their tolerogenic anti-inflammatory properties, MHC-II molecule levels on all LNSCs types are inducible by pro-inflammatory microenvironment in viral infections [108]. Interestingly, lymphoid epithelial cells do express costimulatory molecules in humans, despite their inhibitory function [109]. Nonetheless, murine lymphoid epithelial cells do not express all specific proteins required for endogenous antigen loading and therefore suspectly serve mainly as antigen pool for dendritic cells which subsequently induce CD4 T-lymphocyte anergy. They can however still promote $CD8^+$ T-lymphocyte tolerance using a MHC-II/LAG-3 (lymphocyte-activation gene 3) pathway [110].

Murine LNSCs display a partially opposite effect on Tregs as they do on effector cells – they are important for Treg preservation without additional proliferation [111]. The results further confirm the importance of LNSCs in preventing autoimmunity and promote immune tolerance.

4.10. Vascular endothelial cells and dermal fibroblasts

Endothelial cells were one of the first non-hematopoietic cells shown to be able to express MHC-II molecules, confirmed by many publications in various organs. Nonetheless, human endothelial cells do not express MHC-II until stimulated. The first known stimulant was lectin phytohemagglutinin [112].

Later, it was confirmed that IFN-γ can also induce MHC-II expression on both vascular endothelial cells and dermal fibroblasts, with proposed pathophysiological function in allograft rejection [113]. However, IFN-γ-affected endothelial cells and dermal fibroblasts differed in their ability to stimulate T-cells. While endothelial cells showed the ability to function as non-classical APCs, dermal fibroblasts remained poor stimulators [114]. Apart from antigen presentation, MHC-II molecules on endothelial cells were proven to play an important role in lymphocyte adhesion on the endothelial surface [115]. A functional role for MHC-II expression in fibroblasts, however, remains unknown.

4.11. Gametes

Speculations about MHC-II content on oocytes and sperm cells have long been a matter of discussion. In humans, for both oocytes as well as sperm cells was agreed to be MHC-II negative. On the other hand, mRNA for MHC-II molecules was found in granulosa cells and in immature precursors of sperm cells [116]. However, a study was published showing that sperm cells of infertile men often express MHC-II antigens, while sperm cells of fertile men do so only very rarely. The expression was demonstrated on both transcriptional and protein levels [117].

On the contrary, the situation in mice is quite different. Murine sperm cells typically express *MHC-II* mRNA as well as the protein product at the surface. MHC-II molecules also seem to be critical for fertilization, namely in the sperm cell/egg recognition [118]. A subsequent study demonstrated that the complex structure of MHC-II molecules is necessary for binding of foreign DNA and could be found on the posterior part of sperm head [119]. These findings opened the door for subsequent research on sperm-cell interactions even in other species. The research brought curious discoveries in the field of sexual selection and cryptic female choice which will be further discussed in chapter 5.2.

4.12. Astrocytes

It may be startling that non-professional APCs (other than microglia) can be also found in immune-privileged sites such as brain. Upregulated MHC-II expression was found in correlation to aging, along with other neuroinflammatory markers of astrocyte and microglia activation in both humans and rats. However, the level of neuroinflammation did not correspond to cognitive function decrease [120].

When unstimulated, rat astrocytes were found to express low levels of MHC-II molecules, insufficient to complete T-cell activation. As an effect of unsufficient stimulation, the reduction of T-cell receptors and hyporeactivity or anergy of T-lymphocytes was observed. These findings implicate the anti-inflammatory role of astrocytes in the absence of other inflammatory agents [121].

However, upon IFN-γ stimulation in pure cultures, in comparison to oligodendrocytes, murine astrocytes were able to express MHC-II molecules and present antigens to T-cells [122]. This ability was not lost even after irreversible demyelination such as in chronic multiple sclerosis lesions [123].

In comparison to microglia, murine activated astrocytes still remain less potent APCs, possibly due to less efficient antigen processing. While microglia can stimulate both Th1 and Th2 immune responses, astrocytes mainly induce Th2 lymphocytes [124]. The above data support the notion that astrocytes have a complex but important function on immunomodulation *in vivo*.

5. Possible functions of non-classical MHC-class-II-expressing cells and their uses

5.1. MHC-II and prenatal development

The atypical or unexpected localization of MHC-II molecules in developing tissues and on non-classical MHC-II expressing cells may hint an additional role of MHC-II in prenatal development. A particularly interesting field of research is the nervous system and surrounding accessory tissues. In addition to microglia, cerebral meningeal cells and cells in the choroid plexus in the lateral ventricle were all capable of expressing MHC-II antigens on their surfaces. The expression was detected in human from the 11th week of gestation, implying an important role in physiological fetal development [125]. At the 11th week of gestation, non-classical APCs were already present on thymic epithelial cells, the spleen, the liver and other stromal or lobular cells as well. It was also suggested that during prenatal development, self-antigens were presented on the MHC-II molecules [126].

In rat embryos, neural stem cells (NSCs) from the forebrain expressed MHC-II in small amounts, while neither of the differentiated cells (neurons, oligodendrocytes and astrocytes) did. The MHC-II expression could be retrieved by IFN-γ, the usage of which induced higher expression in NSCs as well [127]. However, IFN-γ treatment produced a differentiation bias in neural stem/progenitor cells in humans. In cultures derived from striatum, the differentiation towards neurons was shifted threefold, while the differentiation towards oligodendrocytes was shifted twofold in cultures from hippocampus[128]. Nonetheless, although IFN-γ treatment increased MHC-II levels on both human NSCs and astrocytes, MHC-II expression on neural progenitors in physiological state seems to be controlled in an IFN-γ-independent manner. As for their basal expression of MHC-II, human NSCs may sometimes cause problems in allogenic embryonic neural cell grafts [129].

While recent findings suggested a distinct role of MHC-II in fetal development, the importance and the exact mechanism of function still remain undetermined.

5.2. MHC-II and sexual selection

The MHC-related sexual selection on any level of reproduction has long been a matter of interest. It is recently believed that one or both genders prefer mating with genetically dissimilar mates, which would bring heterozygosity in offspring, but the explicit role of MHC-related diversity between mates on sexual preference seems difficult to study. Often, the reason behind finding an MHC-dissimilar mate seems to be in better coping with parasites.

To date, most studies investigating the sexual selection have focused on the whole MHC region, not MHC-II alone. If the study differentiated between MHC-I and MHC-II genes, the results were often insignificant or mixed, as it appears to be hard to differentiate MHC-II-related effects from effects of

other alleles in the genome. Additionally, the level of inbreeding often correlates with MHC-II similarity. In Chinook salmon, *Oncorhynchus tsawytscha*, MHC-I diversity appears to be beneficial for its carriers, while MHC-II-homogenous populations were frequent and diversity seemed to be unfavorable [130]. On a cellular level, male MHC-II similarity to a female positively correlated with fertilization success. The surprising results might be explained by the idea that MHC-II is responsible for local adaptation, as Chinook salmon returns to the freshwater of birth upon reproduction. The effect of the male/female MHC-II similarity acts independently from the sperm velocity. The underlying mechanism is proposed to be via protein sperm-egg interactions in cryptic female choice [131].

It is well-known that the results of sexual preference or cryptic choice differ among various species, as distinct mating strategies are present. Further research is needed to evaluate a specific role of MHC-II molecules in both known and unknown selection systems in different species and under different conditions.

5.3. MHC-II and the gut

A role of the MHC-II polymorphism in the diversity and the exact content of microbiome has long been hypothesized. A recent study showed that in three-spined stickleback, about 10 percent of the variability among microflora of individuals could be assigned to MHC-IIb polymorphism, although a gender/MHC association was uncovered. The higher individual MHC-II diversity led to a less diverse microbe composition in the gut [132]. Additional research in this field could correlate these results to other species and find implications for human gastrointestinal and moreover for civilization diseases such as obesity. The exact mechanism by which MHC-II could manipulate bacterial survival in the gut remains an important topic for further research.

The impact of ILCs on inflammatory bowel disease has already been mentioned shortly in chapter 3.3. However, ILCs are not the only non-classical APCs influencing the intestinal homeostasis. As noted briefly in chapter 4.4., an increasing evidence has accumulated demonstrating a huge impact of IECs on the pro- and anti-inflammatory balance. The changes present in various disorders, such as celiac disease or IBDs, have a molecular counterpart in MHC-II expression by IECs, compared to physiological anti-inflammatory state.

To further uncover the role of IECs in physiological immune responses, their functional interactions with two classes of T-cells, namely intestinal epithelial lymphocytes and lamina propria lymphocytes, was studied. IECs were shown to solely activate $CD4^+$ intestinal epithelial lymphocytes, leading to an increase in IFN-γ production [133]. Nevertheless, IECs generally exhibited an anti-inflammatory phenotype, with the activation and proliferation of Tregs [134]. This is consistent with previous findings where the lack of costimulatory molecules resulted in the absence of pro-inflammatory phenotype in the gut. IECs were sufficient for Treg stimulation even after DC reduction, proposing a new, DC-independent pathway of Treg activation [135].

In immunopathologies, such as celiac disease, MHC-II expression on IECs in crypts was induced, as opposed to villus-restricted expression in a physiological state. This is true for patients with active celiac disease or in relapse, but not for patients in remission [136]. Treated, but not fully recovered patients had intermediate levels of MHC-II expression [137]. Gliadin only was found to be a sufficient inducer of MHC-II expression in the crypts of treated celiac disease patients, while also possibly lowering the level of expression on the villi [138]. However, the translocation of gliadin molecules into MHC-II-positive vacuoles was reported only in the jejunal epithelium of untreated celiac patients. Patients treated with a gluten-free diet showed no translocation of gliadin into jejunal epithelial cells [139]. These findings imply the importance of IECs in the pathophysiology of celiac disease in an active inflammation state and also the importance of treatment on abnormal antigen presentation.

In IBDs, more specifically in Crohn's disease, the expression of MHC-II was augmented not only in enterocytes, but also in glial cells and endothelial cells of veins, venules and capillaries. However, as opposed to other cell types, glial cells did not co-express the invariant chain (Ii) [140]. Contrarily, in healthy small intestine Ii was expressed without the co-expression of MHC-II in the IECs of the crypts and in the arterial endothelial cells, while the villous IECs lacked Ii expression while being MHC-II-positive [141]. Upon antigen presentation in IBDs, substantial $CD4^+$ T-cell activation was present, resulting in the IFN-γ production. This contrasted with only subtle $CD4^+$ T-cell activation in normal mucosa [142]. However, by inducing MHC-II on epithelial cells by IFN-γ in a mouse strain otherwise deficient in MHC-II expression on IECs, an anti-inflammatory effect of IFN-γ was shown. After the MHC-II was induced in murine IECs, the colitis symptoms weakened due to partially normalized ratio between proinflammatory Th1 cells and Tregs. Given informations change the view about the IFN-γ as a strictly pro-inflammatory cytokine. The study also helps to understand the reason why anti-IFN-γ-therapy may be insufficient in treating IBDs, even though IBDs are linked to raised levels of IFN-γ [143]. A new approach seems needed to be taken for an efficient treatment of IBDs in the future, possibly manipulating MHC-II-linked antigen presentation.

5.4. MHC-II and HIV

HIV-1 (human immunodeficiency virus 1) was detected to interfere with MHC-II expression. Tat, a viral transactivator protein, inhibits CIITA expression in APCs and vice versa by the competition for a cofactor, cyclin T1 [144]. Depending on the prevailing effector, the result could be either inhibition or supression of viral replication by CIITA upregulation (even in T-cells) [145] or the reduction of *MHC-II* transcription by the repression of CIITA function [146]. However, Tat was able to prevent *MHC-II* transcription even in murine model where Tat does not interact functionally with cyclin T1, suggesting an additional mechanism in CIITA repression [146]. By CIITA over-expression *in vivo*, a novel therapeutical mechanism for treating HIV-1 infection and AIDS may be possible.

6. Summary

MHC-II is frequently expressed on other cell types apart from professional APCs. Both constitutive and facultative expression was observed, the facultative one often being in response to inflammatory stimuli (the most prevalent being IFN-γ). To the already mentioned examples we can add the fibroblast-like synoviocytes expressing MHC-II in postinfectious Lyme arthritis [147] and the esophageal epithelial cells in Eosinophilic Esophagitis [148].

One of the most peculiar sites of MHC-II expression are the immune-privileged sites, such as the eye and the brain. The expression here is observed upon simultaneous IFN-γ and TNF-α stimulation in a CIITA-independent manner. However, the cells seem to present endogenous rather than exogenous antigens and the MHC-II expression may be the reason of rejecting corneal allografts [149].

However, MHC-II expression does not equal T-cell stimulation, as the costimulatory molecules are often not expressed. Furthermore, the data obtained by a single method are often unsatisfactory for proving MHC-II gene expression. Just as detected mRNA does not always mean protein expression on the cell surface thanks to mRNA abrogation or post-transcriptional protein degradation, immunohistochemical detection of surface protein does not always mean gene expression. This is due to a process called trogocytosis, mentioned earlier in the text, which enables cells to gain patches of cytoplasm from other cells and thus express MHC-II on their surfaces. Examples of this process have been reported in murine T-cells, basophils, LNSCs and other [35,55,106]. Another way of gaining MHC-II molecules is through exosomes, small vesicles secreted by several cell types, such as IECs [86] or via nanotubes. To conclude, various methods should always be used to ensure *MHC-II* gene expression on non-classical MHC-II-expressing cells.

Various aspects of MHC-II expression are further summarized in Table 1.

Table 1: Summary of MHC-II expression by various cell types. Abbreviations:, devel – development, diff. – differentiation, EC – endothelial cell, IEC – intestinal epithelial cell, ILC – innate lymphoid cell, ISC – intestinal stem cell, LNSC – lymph node stromal cell, n.m. – not mentioned, TEC – thymic epithelial cell.

Cell type	**Constitutive MHC-II expression**	**Observed in humans**	**Observed in mice**	**Can serve as APC**	**Costimulatory molecule expression**	**Other functions**
Hematopoietic progenitors	some	yes	yes	yes	n.m.	diff. bias
T-cells	some	yes	yes (acquired)	yes	murine (acquired), otherwise n.m.	
ILCs	some	yes	yes	yes	some	IBDs

Basophils	mixed results	yes	yes	yes	n.m.	
Eosinophils	no	yes	n.m.	yes	yes	
Neutrophils	no	yes	n.m.	mixed results	yes	
Thyroid cells	no	yes	n.m.	n.m.	n.m.	
Pancreatic islet cells	no	n.m.	yes	yes	no	
Hepatocytes	no	yes	yes	yes	yes	
IECs	yes	yes	yes	yes	sometimes	IBDs, celiac disease
Bronchial epithelial cells	mixed	yes	n.m.	some-times	sometimes	
Pneumocytes type II	yes	yes	n.m.	some-times	sometimes	
ISCs	yes (but elevated in infections)	n.m.	yes	yes	n.m.	Self-renewal vs. diff.
Subepithelial myofibroblasts	yes	yes	n.m.	yes	yes	
TECs	yes	yes	yes	yes	n.m.	
LNSCs	yes	yes	yes	yes	yes	
Vascular ECs	no	yes	n.m.	yes	n.m.	
Dermal fibroblasts	no	yes	n.m.	no	n.m.	
Gametes (sperm cells)	depends on species	yes (sometimes)	yes	n.m.	n.m.	Sexual selection
Astrocytes	no	yes	yes	yes	n.m.	Prenatal devel.
Cell type	**Constitutive MHC-II expression**	**Observed in humans**	**Observed in mice**	**Can serve as APC**	**Costimulatory molecule expression**	**Other functions**

7. Conclusion

Major histocompatibility complex class II molecules have been extensively studied ever since their discovery. The convention that only professional antigen-presenting cells bear the ability to express MHC-II molecules was questioned soon after its origin. Gathering evidence has already proven the inaccuracy of such predictions and demonstrated the nearly universal capacity of cells of both hematopoietic and non-hematopoietic origin to express MHC-II molecules. Numerous studies confirmed the endogenous origin of MHC-II molecules in the most cases and clarified the exact molecular mechanism and stimuli critical for MHC-II expression. However, under specific conditions, some cellular types can also acquire exogenous MHC-II molecules via trogocytosis, exosomes or nanotubes.

The functions of non-classical MHC-II expression on various cell types vary from the „typical“ function of antigen presentation, with the outcomes ranging from T-cell activation to anergy and apoptosis, to a seemingly non-immunological role. The examples of these roles include prenatal development or sexual selection, including cryptic choice. However, the amount of discovered functions is continuously growing and for some cells, a significant function of MHC-II expression is still undetermined.

There are many unanswered questions in this field of study and further research should help address them and evaluate obtained results.

8. References

1. Cresswell P. Invariant chain structure and MHC class II function. *Cell*. 1996;84(4):505-507.
2. Sanderson F, Trowsdale J. Antigen presentation: Kissing cousins exchange CLIP. *Curr Biol*. 1995;5(12):1372-1376.
3. Chang CH, Fontes JD, Peterlin M, Flavell RA. Class II transactivator (CIITA) is sufficient for the inducible expression of major histocompatibility complex class II genes. *J Exp Med*. 1994;180(4):1367-1374.
4. Ting JP-Y, Trowsdale J. Genetic control of MHC class II expression. *Cell*. 2002;109 Suppl(2):S21-33. Review.
5. Fan Z, Li J, Li P, et al. Protein arginine methyltransferase 1 (PRMT1) represses MHC II transcription in macrophages by methylating CIITA. *Sci Rep*. 2017;7(1):40531.
6. Bissa M, Forlani G, Zanotto C, et al. Fowlpoxvirus recombinants coding for the CIITA gene increase the expression of endogenous MHC-II and Fowlpox Gag/Pro and Env SIV transgenes. *PLoS One*. 2018;13(1):e0190869.
7. Beaulieu YB, Leon Machado JA, Ethier S, Gaudreau L, Steimle V. Degradation, Promoter Recruitment and Transactivation Mediated by the Extreme N-Terminus of MHC Class II Transactivator CIITA Isoform III. *PLoS One*. 2016;11(2):e0148753.
8. Muhlethaler-Mottet A, Otten LA, Steimle V, Mach B. Expression of MHC class II molecules in different cellular and functional compartments is controlled by differential usage of multiple promoters of the transactivator CIITA. *EMBO J*. 1997;16(10):2851-2860.
9. Piskurich JF, Gilbert CA, Ashley BD, et al. Expression of the MHC class II transactivator (CIITA) type IV promoter in B lymphocytes and regulation by IFN-gamma. *Mol Immunol*. 2006;43(6):519-528.
10. Lee MS, Lee JO, Kang TW, Lee JH. Transcription of class II MHC gene by interferon-gamma in FRTL-5 cells. *J Korean Med Sci*. 1992;7(3):189.
11. Muhlethaler-Mottet A, Di Berardino W, Otten LA, Mach B. Activation of the MHC class II transactivator CIITA by interferon-gamma requires cooperative interaction between Stat1 and USF-1. *Immunity*. 1998;8(2):157-166.
12. Waldburger J-M, Suter T, Fontana A, Acha-Orbea H, Reith W. Selective Abrogation of Major Histocompatibility Complex Class II Expression on Extrahematopoietic Cells in Mice Lacking Promoter IV of the Class II Transactivator Gene. *J Exp Med*. 2001;194(4):393.
13. Lee YJ, Panek RB, Huston M, Benveniste EN. Role of protein kinase C and tyrosine kinase activity in IFN-gamma-induced expression of the class II MHC gene. *Am J Physiol Physiol*. 1995;268(1):C127-C137.
14. Benveniste EN, Vidovic M, Panek RB, Norris JG, Reddy AT, Benos DJ. Interferon-gamma-induced astrocyte class II major histocompatibility complex gene expression is associated with

both protein kinase C activation and Na+ entry. *J Biol Chem*. 1991;266(27):18119-18126.

15. Smith MR, Ramsburg EA, Kung HF, Durum SK. Components of the protein kinase C pathway induce Ia expression after injection into macrophages. *J Immunol*. 1992;149(4):1304-1310.
16. Ivaska J, Bosca L, Parker PJ. PKCε is a permissive link in integrin-dependent IFN-γ signalling that facilitates JAK phosphorylation of STAT1. *Nat Cell Biol*. 2003;5(4):363-369.
17. Giroux M, Schmidt M, Descoteaux A. IFN-gamma-induced MHC class II expression: transactivation of class II transactivator promoter IV by IFN regulatory factor-1 is regulated by protein kinase C-alpha. *J Immunol*. 2003;171(8):4187-4194.
18. Kwon M-J, Yao Y, Walter MJ, Holtzman MJ, Chang C-H. Role of PKCδ in IFN-γ-inducible CIITA gene expression. *Mol Immunol*. 2007;44(11):2841.
19. Watson CA, Petzelbauer P, Zhou J, Pardi R, Bender JR. Contact-dependent endothelial class II HLA gene activation induced by NK cells is mediated by IFN-gamma-dependent and -independent mechanisms. *J Immunol*. 1995;154(7):3222-3233.
20. Collinge M, Pardi R, Bender JR. Class II transactivator-independent endothelial cell MHC class II gene activation induced by lymphocyte adhesion. *J Immunol*. 1998;161(4):1589-1593.
21. Chirathaworn C, Tibbetts SA, Chan MA, Benedict SH. Cross-linking of ICAM-1 on T cells induces transient tyrosine phosphorylation and inactivation of cdc2 kinase. *J Immunol*. 1995;155(12):5479-5482.
22. Durieu-Trautmann O, Chaverot N, Cazaubon S, Strosberg AD, Couraud PO. Intercellular adhesion molecule 1 activation induces tyrosine phosphorylation of the cytoskeleton-associated protein cortactin in brain microvessel endothelial cells. *J Biol Chem*. 1994;269(17):12536-12540.
23. Gunzer M, Schäfer A, Borgmann S, et al. Antigen presentation in extracellular matrix: interactions of T cells with dendritic cells are dynamic, short lived, and sequential. *Immunity*. 2000;13(3):323-332.
24. König R, Huang L-Y, Germain RN. MHC class II interaction with CD4 mediated by a region analogous to the MHC class I binding site for CD8. *Nature*. 1992;356(6372):796-798.
25. Bodger M, Izaguirre C, Blacklock H, Hoffbrand A. Surface antigenic determinants on human pluripotent and unipotent hematopoietic progenitor cells. *Blood*. 1983;61(5):1006-1010.
26. Finkielsztein A, Schlinker AC, Zhang L, Miller WM, Datta SK. Human megakaryocyte progenitors derived from hematopoietic stem cells of normal individuals are MHC class II-expressing professional APC that enhance Th17 and Th1/Th17 responses. *Immunol Lett*. 2015;163(1):84-95.
27. Bertho N, Drénou B, Mooney N, et al. HLA-DR inhibits granulocytic differentiation without inducing apoptosis of CD34 cells. *Hum Immunol*. 1999;60(10):944-954.
28. Urbieta M, Barao I, Jones M, et al. Hematopoietic progenitor cell regulation by CD4+CD25+ T cells. *Blood*. 2010;115(23):4934-4943.

29. Hayakawa K, Tarlinton D, Hardy RR. Absence of MHC class II expression distinguishes fetal from adult B lymphopoiesis in mice. *J Immunol*. 1994;152(10):4801-4807.
30. Ko HS, Fu SM, Winchester RJ, Yu DT, Kunkel HG. Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen- and antigen-activated T cells. *J Exp Med*. 1979;150(2):246-255.
31. Holling TM, van der Stoep N, Quinten E, van den Elsen PJ. Activated human T cells accomplish MHC class II expression through T cell-specific occupation of class II transactivator promoter III. *J Immunol*. 2002;168(2):763-770.
32. Baecher-Allan C, Wolf E, Hafler DA. MHC class II expression identifies functionally distinct human regulatory T cells. *J Immunol*. 2006;176(8):4622-4631.
33. Chang CH, Hong SC, Hughes CC, Janeway CA, Flavell RA. CIITA activates the expression of MHC class II genes in mouse T cells. *Int Immunol*. 1995;7(9):1515-1518.
34. Holling TM, Schooten E, Langerak AW, van den Elsen PJ. Regulation of MHC class II expression in human T-cell malignancies. *Blood*. 2003;103(4):1438-1444.
35. Tsang JYS, Chai JG, Lechler R. Antigen presentation by mouse CD4+ T cells involving acquired MHC class II:peptide complexes: another mechanism to limit clonal expansion? *Blood*. 2003;101(7):2704-2710.
36. Crepaldi T, Crump A, Newman M, Ferrone S, Antczak DF. Equine T lymphocytes express MHC class II antigens. *J Immunogenet*. 1986;13(4):349-360.
37. Lunn DP, Holmes MA, Duffus WP. Equine T-lymphocyte MHC II expression: variation with age and subset. *Vet Immunol Immunopathol*. 1993;35(3-4):225-238.
38. Barbis DP, Bainbridge D, Crump AL, Zhang CH, Antczak DF. Variation in expression of MHC class II antigens on horse lymphocytes determined by MHC haplotype. *Vet Immunol Immunopathol*. 1994;42(1):103-114.
39. Saalmüller A, Weiland F, Reddehase MJ. Resting Porcine T Lymphocytes Expressing Class II Major Histocompatibility Antigen. *Immunobiology*. 1991;183(1-2):102-114.
40. Saalmüller A, Maurer S. Major Histocompatibility Antigen Class II Expressing Resting Porcine T Lymphocytes are Potent Antigen-Presenting Cells in Mixed Leukocyte Culture. *Immunobiology*. 1994;190(1-2):23-34.
41. Summerfield A, Rziha H-J, Saalmüller A. Functional Characterization of Porcine CD4+CD8+Extrathymic T Lymphocytes. *Cell Immunol*. 1996;168(2):291-296.
42. Eberl G, Colonna M, Di Santo JP, McKenzie ANJ. Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology. *Science*. 2015;348(6237):aaa6566. Review.
43. Mirchandani AS, Besnard A-G, Yip E, et al. Type 2 Innate Lymphoid Cells Drive CD4+ Th2 Cell Responses. *J Immunol*. 2014;192(5):2442-2448.
44. Mebius RE, Rennert P, Weissman IL. Developing Lymph Nodes Collect CD4+CD3− LTβ+ Cells That Can Differentiate to APC, NK Cells, and Follicular Cells but Not T or B Cells.

Immunity. 1997;7(4):493-504.

45. Hepworth MR, Monticelli LA, Fung TC, et al. Innate lymphoid cells regulate CD4+ T-cell responses to intestinal commensal bacteria. *Nature*. 2013;498(7452):113-117.
46. Hepworth MR, Fung TC, Masur SH, et al. Immune tolerance. Group 3 innate lymphoid cells mediate intestinal selection of commensal bacteria-specific $CD4^+$ T cells. *Science*. 2015;348(6238):1031-1035.
47. Salimi M, Wang R, Yao X, et al. Activated innate lymphoid cell populations accumulate in human tumour tissues. *BMC Cancer*. 2018;18(1):341.
48. Yoshimoto T, Yasuda K, Tanaka H, et al. Basophils contribute to TH2-IgE responses in vivo via IL-4 production and presentation of peptide–MHC class II complexes to CD4+ T cells. *Nat Immunol*. 2009;10(7):706-712.
49. Sokol CL, Chu N-Q, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol*. 2009;10(7):713-720.
50. Perrigoue JG, Saenz SA, Siracusa MC, et al. MHC class II-dependent basophil-CD4+ T cell interactions promote T(H)2 cytokine-dependent immunity. *Nat Immunol*. 2009;10(7):697-705.
51. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. *Nat Immunol*. 2008;9(3):310-318.
52. Sharma M, Hegde P, Aimanianda V, et al. Circulating human basophils lack the features of professional antigen presenting cells. *Sci Rep*. 2013;3:1188.
53. Poulsen BC, Poulsen LK, Jensen BM. Detection of MHC class II expression on human basophils is dependent on antibody specificity but independent of atopic disposition. *J Immunol Methods*. 2012;381(1-2):66-69.
54. Voskamp AL, Prickett SR, Mackay F, Rolland JM, O'Hehir RE. MHC class II expression in human basophils: induction and lack of functional significance. *PLoS One*. 2013;8(12):e81777.
55. Miyake K, Shiozawa N, Nagao T, Yoshikawa S, Yamanishi Y, Karasuyama H. Trogocytosis of peptide-MHC class II complexes from dendritic cells confers antigen-presenting ability on basophils. *Proc Natl Acad Sci U S A*. 2017;114(5):1111-1116.
56. Lucey DR, Nicholson-Weller A, Weller PF. Mature human eosinophils have the capacity to express HLA-DR. *Proc Natl Acad Sci U S A*. 1989;86(4):1348-1351.
57. Hansel TT, Braunstein JB, Walker C, et al. Sputum eosinophils from asthmatics express ICAM-1 and HLA-DR. *Clin Exp Immunol*. 1991;86(2):271-277.
58. Jung Y-J, Woo S-Y, Jang MH, et al. Human Eosinophils Show Chemotaxis to Lymphoid Chemokines and Exhibit Antigen-Presenting-Cell-Like Properties upon Stimulation with IFN-γ, IL-3 and GM-CSF. *Int Arch Allergy Immunol*. 2008;146(3):227-234.
59. Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha

expression. *J Immunol*. 1993;150(6):2554-2562.

60. Hansel TT, De Vries IJ, Carballido JM, et al. Induction and function of eosinophil intercellular adhesion molecule-1 and HLA-DR. *J Immunol*. 1992;149(6):2130-2136.
61. Gosselin EJ, Wardwell K, Rigby WF, Guyre PM. Induction of MHC class II on human polymorphonuclear neutrophils by granulocyte/macrophage colony-stimulating factor, IFN-gamma, and IL-3. *J Immunol*. 1993;151(3):1482-1490.
62. Vono M, Lin A, Norrby-Teglund A, Koup RA, Liang F, Loré K. Neutrophils acquire the capacity for antigen presentation to memory CD4+ T cells in vitro and ex vivo. *Blood*. 2017;129(14):1991.
63. Abi Abdallah DS, Egan CE, Butcher BA, Denkers EY. Mouse neutrophils are professional antigen-presenting cells programmed to instruct Th1 and Th17 T-cell differentiation. *Int Immunol*. 2011;23(5):317-326.
64. Hanafusa T, Pujol-Borrell R, Chiovato L, Russell RC, Doniach D, Bottazzo GF. Aberrant expression of HLA-DR antigen on thyrocytes in Graves' disease: relevance for autoimmunity. *Lancet (London, England)*. 1983;2(8359):1111-1115.
65. Piccinini LA, Goldsmith NK, Roman SH, Davies TF. HLA-DP, DQ and DR gene expression in Graves' disease and normal thyroid epithelium. *Tissue Antigens*. 1987;30(4):145-154.
66. Platzer M, Neufeld DS, Piccini LA, Davies TF. Induction of Rat Thyroid Cell MHC Class II Antigen by Thyrotropin and Y-Interferon*. *Endocrinology*. 1987;121(6):2087-2092.
67. Zhao Y, Scott NA, Quah HS, et al. Mouse pancreatic beta cells express MHC class II and stimulate CD4 $^{+}$ T cells to proliferate. *Eur J Immunol*. 2015;45(9):2494-2503.
68. Scott NA, Zhao Y, Krishnamurthy B, Mannering SI, Kay TWH, Thomas HE. IFNγ-Induced MHC Class II Expression on Islet Endothelial Cells Is an Early Marker of Insulitis but Is Not Required for Diabetogenic CD4+ T Cell Migration. *Front Immunol*. 2018;9:2800.
69. Dienes HP, Hütteroth T, Hess G, Meuer SC. Immunoelectron microscopic observations on the inflammatory infiltrates and HLA antigens in hepatitis B and non-A, non-B. *Hepatology*. 1987;7(6):1317-1325.
70. Franco A, Barnaba V, Natali P, Balsano C, Musca A, Balsano F. Expression of class I and class II major histocompatibility complex antigens on human hepatocytes. *Hepatology*. 1988;8(3):449-454.
71. Doherty DG, Donaldson PT, Underhill JA, et al. Allelic sequence variation in the HLA class II genes and proteins in patients with autoimmune hepatitis. *Hepatology*. 1994;19(3):609-615.
72. Yamagiwa S, Kamimura H, Takamura M, et al. Presence of antibodies against self human leukocyte antigen class II molecules in autoimmune hepatitis. *Int J Med Sci*. 2014;11(9):850-856.
73. Bexfield NH, Watson PJ, Aguirre-Hernandez J, et al. DLA class II alleles and haplotypes are associated with risk for and protection from chronic hepatitis in the English Springer spaniel.

PLoS One. 2012;7(8):e42584.

74. Herkel J, Jagemann B, Wiegard C, et al. MHC class II-expressing hepatocytes function as antigen-presenting cells and activate specific CD4 T lymphocytes. *Hepatology*. 2003;37(5):1079-1085.
75. Kim H, Mazumdar B, Bose SK, et al. Hepatitis C virus-mediated inhibition of cathepsin S increases invariant-chain expression on hepatocyte surface. *J Virol*. 2012;86(18):9919-9928.
76. Wiegard C, Wolint P, Frenzel C, et al. Defective T Helper Response of Hepatocyte-Stimulated CD4 T Cells Impairs Antiviral CD8 Response and Viral Clearance. *Gastroenterology*. 2007;133(6):2010-2018.
77. Daar AS, Fuggle S V, Fabre JW, Ting A, Morris PJ. The detailed distribution of MHC Class II antigens in normal human organs. *Transplantation*. 1984;38(3):293-298.
78. Scott H, Solheim BG, Brandtzaeg P, Thorsby E. HLA-DR-like antigens in the epithelium of the human small intestine. *Scand J Immunol*. 1980;12(1):77-82.
79. MacDonald TT, Weinel A, Spencer J. HLA-DR expression in human fetal intestinal epithelium. *Gut*. 1988;29(10):1342-1348.
80. Nagura H, Ohtani H, Masuda T, Kimura M, Nakamura S. HLA-DR expression on M cells overlying Peyer's patches is a common feature of human small intestine. *Acta Pathol Jpn*. 1991;41(11):818-823.
81. Hershberg RM, Cho DH, Youakim A, et al. Highly polarized HLA class II antigen processing and presentation by human intestinal epithelial cells. *J Clin Invest*. 1998;102(4):792-803.
82. Byrne B, Madrigal-Estebas L, McEvoy A, et al. Human duodenal epithelial cells constitutively express molecular components of antigen presentation but not costimulatory molecules. *Hum Immunol*. 2002;63(11):977-986.
83. Nakazawa A, Watanabe M, Kanai T, et al. Functional expression of costimulatory molecule CD86 on epithelial cells in the inflamed colonic mucosa. *Gastroenterology*. 1999;117(3):536-545.
84. Borcherding F, Nitschke M, Hundorfean G, et al. The CD40-CD40L pathway contributes to the proinflammatory function of intestinal epithelial cells in inflammatory bowel disease. *Am J Pathol*. 2010;176(4):1816-1827.
85. Sanderson IR, Ouellette AJ, Carter EA, Walker WA, Harmatz PR. Differential regulation of B7 mRNA in enterocytes and lymphoid cells. *Immunology*. 1993;79(3):434-438.
86. van Niel G, Raposo G, Candalh C, et al. Intestinal epithelial cells secrete exosome-like vesicles. *Gastroenterology*. 2001;121(2):337-349.
87. Mallegol J, Van Niel G, Lebreton C, et al. T84-Intestinal Epithelial Exosomes Bear MHC Class II/Peptide Complexes Potentiating Antigen Presentation by Dendritic Cells. *Gastroenterology*. 2007;132(5):1866-1876.
88. Van Niel G, Mallegol J, Bevilacqua C, et al. Intestinal epithelial exosomes carry MHC class

II/peptides able to inform the immune system in mice. *Gut*. 2003;52(12):1690-1697.

89. Karlsson M, Lundin S, Dahlgren U, Kahu H, Pettersson I, Telemo E. "Tolerosomes" are produced by intestinal epithelial cells. *Eur J Immunol*. 2001;31(10):2892-2900.
90. Glanville AR, Tazelaar HD, Theodore J, et al. The Distribution of MHC Class I and II Antigens on Bronchial Epithelium. *Am Rev Respir Dis*. 1989;139(2):330-334.
91. Rossi GA, Sacco O, Balbi B, et al. Human Ciliated Bronchial Epithelial Cells: Expression of the HLA-DR Antigens and of the HLA-DR Alpha Gene, Modulation of the HLA-DR Antigens by Gamma-Interferon and Antigen-presenting Function in the Mixed Leukocyte Reaction. *Am J Respir Cell Mol Biol*. 1990;3(5):431-439.
92. Vignola AM, Campbell AM, Chanez P, et al. HLA-DR and ICAM-1 Expression on Bronchial Epithelial Cells in Asthma and Chronic Bronchitis. *Am Rev Respir Dis*. 1993;148(3):689-694.
93. Suda T, Sato A, Sugiura W, Chida K. Induction of MHC class II antigens on rat bronchial epithelial cells by interferon-gamma and its effect on antigen presentation. *Lung*. 1995;173(2):127-137.
94. Cunningham AC, Milne DS, Wilkes J, Dark JH, Tetley TD, Kirby JA. Constitutive expression of MHC and adhesion molecules by alveolar epithelial cells (type II pneumocytes) isolated from human lung and comparison with immunocytochemical findings. *J Cell Sci*. 1994;107 (Pt 2):443-449.
95. Cunningham AC, Zhang JG, Moy J V, Ali S, Kirby JA. A comparison of the antigen-presenting capabilities of class II MHC-expressing human lung epithelial and endothelial cells. *Immunology*. 1997;91(3):458-463.
96. Ibrahim L, Dominguez M, Yacoub M. Primary human adult lung epithelial cells in vitro: response to interferon-gamma and cytomegalovirus. *Immunology*. 1993;79(1):119-124.
97. Kaneko Y, Kuwano K, Kunitake R, Kawasaki M, Hagimoto N, Hara N. B7-1, B7-2 and class II MHC molecules in idiopathic pulmonary fibrosis and bronchiolitis obliterans-organizing pneumonia. *Eur Respir J*. 2000;15(1):49-55.
98. Elssner A, Jaumann F, Wolf WP, et al. Bronchial epithelial cell B7-1 and B7-2 mRNA expression after lung transplantation: a role in allograft rejection? *Eur Respir J*. 2002;20(1):165-169.
99. Biton M, Haber AL, Rogel N, et al. T Helper Cell Cytokines Modulate Intestinal Stem Cell Renewal and Differentiation. *Cell*. 2018;175(5):1307-1320.e22.
100. Saada JI, Pinchuk I V, Barrera CA, et al. Subepithelial myofibroblasts are novel nonprofessional APCs in the human colonic mucosa. *J Immunol*. 2006;177(9):5968-5979.
101. Laufer TM, DeKoning J, Markowitz JS, Lo D, Glimcher LH. Unopposed positive selection and autoreactivity in mice expressing class II MHC only on thymic cortex. *Nature*. 1996;383(6595):81-85.
102. Benoist C, Mathis D. Positive selection of the T cell repertoire: where and when does it occur?

Cell. 1989;58(6):1027-1033.

103. Anderson G, Jenkinson EJ, Moore NC, Owen JJT. MHC class II-positive epithelium and mesenchyme cells are both required for T-cell development in the thymus. *Nature*. 1993;362(6415):70-73.
104. Witherden D, Oers N van, Waltzinger C, Weiss A, Benoist C, Mathis D. Tetracycline-controllable Selection of CD4+ T Cells: Half-Life and Survival Signals in the Absence of Major Histocompatibility Complex Class II Molecules. *J Exp Med*. 2000;191(2):355-364.
105. Waldburger J-M, Rossi S, Hollander GA, Rodewald H-R, Reith W, Acha-Orbea H. Promoter IV of the class II transactivator gene is essential for positive selection of CD4+ T cells. *Blood*. 2003;101(9):3550-3559.
106. Dubrot J, Duraes F V, Potin L, et al. Lymph node stromal cells acquire peptide-MHCII complexes from dendritic cells and induce antigen-specific $CD4^+$ T cell tolerance. *J Exp Med*. 2014;211(6):1153-1166.
107. Dubrot J, Duraes F V, Harlé G, et al. Absence of MHC-II expression by lymph node stromal cells results in autoimmunity. *Life Sci Alliance*. 2018;1(6): e201800164.
108. Abe J, Shichino S, Ueha S, et al. Lymph node stromal cells negatively regulate antigen-specific CD4+ T cell responses. *J Immunol*. 2014;193(4):1636-1644.
109. Nörder M, Gutierrez MG, Zicari S, Cervi E, Caruso A, Guzmán CA. Lymph node-derived lymphatic endothelial cells express functional costimulatory molecules and impair dendritic cell-induced allogenic T-cell proliferation. *FASEB J*. 2012;26(7):2835-2846.
110. Rouhani SJ, Eccles JD, Riccardi P, et al. Roles of lymphatic endothelial cells expressing peripheral tissue antigens in CD4 T-cell tolerance induction. *Nat Commun*. 2015;6:6771.
111. Baptista AP, Roozendaal R, Reijmers RM, et al. Lymph node stromal cells constrain immunity via MHC class II self-antigen presentation. *Elife*. 2014;3:e04433.
112. Pober JS, Gimbrone Jr MA. Expression of Ia-like antigens by human vascular endothelial cells is inducible in vitro: demonstration by monoclonal antibody binding and immunoprecipitation. *Proc Natl Acad Sci U S A*. 1982;79(21):6641-6645.
113. Pober JS, Collins T, Gimbrone MA, et al. Lymphocytes recognize human vascular endothelial and dermal fibroblast Ia antigens induced by recombinant immune interferon. *Nature*. 1983;305(5936):726-729.
114. Geppert TD, Lipsky PE. Antigen presentation by interferon-gamma-treated endothelial cells and fibroblasts: differential ability to function as antigen-presenting cells despite comparable Ia expression. *J Immunol*. 1985;135(6):3750-3762.
115. Masuyama J, Minato N, Kano S. Mechanisms of lymphocyte adhesion to human vascular endothelial cells in culture. T lymphocyte adhesion to endothelial cells through endothelial HLA-DR antigens induced by gamma interferon. *J Clin Invest*. 1986;77(5):1596.
116. García-Láez V, del los Santos Molina JM, Serra V, Pellicer A, Santos M, De los Santos Molina

JM. Expression of Major Histocompatibility Complex (MHC) class I and class II on human gametes. *Revista Iberoamericana de Fertilidad y Reproduccion Humana.* 2008;25:333-344.

117. Paradisi R, Neri S, Pession A, et al. Human leukocyte antigen II expression in sperm cells: comparison between fertile and infertile men. *Arch Androl*. 2000;45(3):203-213.
118. Mori T, Wu GM, Mori E, et al. Expression of class II major histocompatibility complex antigen on mouse sperm and its roles in fertilization. *Am J Reprod Immunol*. 1990;24(1):9-14.
119. Wu GM, Nose K, Mori E, Mori T. Binding of foreign DNA to mouse sperm mediated by its MHC class II structure. *Am J Reprod Immunol*. 1990;24(4):120-126.
120. VanGuilder HD, Bixler G V, Brucklacher RM, et al. Concurrent hippocampal induction of MHC II pathway components and glial activation with advanced aging is not correlated with cognitive impairment. *J Neuroinflammation*. 2011;8:138.
121. Sun D, Coleclough C, Whitaker JN. Nonactivated astrocytes downregulate T cell receptor expression and reduce antigen-specific proliferation and cytokine production of myelin basic protein (MBP)-reactive T cells. *J Neuroimmunol*. 1997;78(1-2):69-78.
122. Takiguchi M, Frelinger JA. Induction of antigen presentation ability in purified cultures of astroglia by interferon-gamma. *J Mol Cell Immunol*. 1986;2(5):269-280.
123. Cannella B, Aquino DA, Raine CS. MHC II expression in the CNS after long-term demyelination. *J Neuropathol Exp Neurol*. 1995;54(4):521-530.
124. Aloisi F, Ria F, Penna G, Adorini L. Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. *J Immunol*. 1998;160(10):4671-4680.
125. Wierzba-Bobrowicz T, Schmidt-Sidor B, Gwiazda E, Lechowicz W, Kosno-Kruszewska E. Major histocompatibility complex class II expression in the frontal and temporal lobes in the human fetus during development. *Folia Neuropathol*. 2000;38(2):73-77.
126. Wierzba-Bobrowicz T, Kosno-Kruszewska E, Gwiazda E, Lechowicz W. Major histocompatibility complex class II (MHC II) expression during the development of human fetal cerebral occipital lobe, cerebellum, and hematopoietic organs. *Folia Neuropathol*. 2000;38(3):111-118.
127. Yin L, Fu S-L, Shi G-Y, et al. Expression and Regulation of Major Histocompatibility Complex on Neural Stem Cells and Their Lineages. *Stem Cells Dev*. 2008;17(1):53-66.
128. Johansson S, Price J, Modo M. Effect of Inflammatory Cytokines on Major Histocompatibility Complex Expression and Differentiation of Human Neural Stem/Progenitor Cells. *Stem Cells*. 2008;26(9):2444-2454.
129. Vagaska B, New SEP, Alvarez-Gonzalez C, et al. MHC-class-II are expressed in a subpopulation of human neural stem cells in vitro in an IFNγ–independent fashion and during development. *Sci Rep*. 2016;6(1):24251.
130. Evans ML, Neff BD, Heath DD. MHC-mediated local adaptation in reciprocally translocated Chinook salmon. *Conserv Genet*. 2010;11(6):2333-2342. doi:10.1007/s10592-010-0119-3

131. Gessner C, Nakagawa S, Zavodna M, Gemmell NJ. Sexual selection for genetic compatibility: the role of the major histocompatibility complex on cryptic female choice in Chinook salmon (Oncorhynchus tshawytscha). *Heredity (Edinb)*. 2017;118(5):442-452.
132. Bolnick DI, Snowberg LK, Caporaso JG, Lauber C, Knight R, Stutz WE. Major Histocompatibility Complex class IIb polymorphism influences gut microbiota composition and diversity. *Mol Ecol*. 2014;23(19):4831-4845.
133. Hatano R, Yamada K, Iwamoto T, et al. Antigen presentation by small intestinal epithelial cells uniquely enhances IFN-γ secretion from CD4+ intestinal intraepithelial lymphocytes. *Biochem Biophys Res Commun*. 2013;435(4):592-596.
134. Westendorf AM, Bruder D, Hansen W, Buer J. Intestinal Epithelial Antigen Induces CD4+ T Cells with Regulatory Phenotype in a Transgenic Autoimmune Mouse Model. *Ann N Y Acad Sci*. 2006;1072(1):401-406.
135. Westendorf AM, Fleissner D, Groebe L, et al. CD4+Foxp3+ regulatory T cell expansion induced by antigen-driven interaction with intestinal epithelial cells independent of local dendritic cells. *Gut*. 2009;58(2):211-219.
136. Arnaud-Battandier F, Cerf-Bensussan N, Amsellem R, Schmitz J. Increased HLA-DR expression by enterocytes in children with celiac disease. *Gastroenterology*. 1986;91(5):1206-1212.
137. Kelly J, O'Farrelly C, O'Mahony C, Weir DG, Feighery C. Immunoperoxidase demonstration of the cellular composition of the normal and coeliac small bowel. *Clin Exp Immunol*. 1987;68(1):177-188.
138. Fais S, Maiuri L, Pallone F, et al. Gliadin induced changes in the expression of MHC-class II antigens by human small intestinal epithelium. Organ culture studies with coeliac disease mucosa. *Gut*. 1992;33(4):472-475.
139. Zimmer KP, Poremba C, Weber P, Ciclitira PJ, Harms E. Translocation of gliadin into HLA-DR antigen containing lysosomes in coeliac disease enterocytes. *Gut*. 1995;36(5):703-709.
140. Koretz K, Momburg F, Otto HF, Möller P. Sequential induction of MHC antigens on autochthonous cells of ileum affected by Crohn's disease. *Am J Pathol*. 1987;129(3):493-502.
141. Momburg F, Koretz K, Von Herbay A, Möller P. Nonimmune human cells can express MHC class II antigens in the absence of invariant chain--an immunohistological study on normal and chronically inflamed small intestine. *Clin Exp Immunol*. 1988;72(3):367-372.
142. Dotan I, Allez M, Nakazawa A, Brimnes J, Schulder-Katz M, Mayer L. Intestinal epithelial cells from inflammatory bowel disease patients preferentially stimulate CD4+ T cells to proliferate and secrete interferon-γ. *Am J Physiol Liver Physiol*. 2007;292(6):G1630-G1640.
143. Thelemann C, Eren RO, Coutaz M, et al. Interferon-γ Induces Expression of MHC Class II on Intestinal Epithelial Cells and Protects Mice from Colitis. *PLoS One*. 2014;9(1):e86844.
144. Okamoto H, Asamitsu K, Nishimura H, Kamatani N, Okamoto T. Reciprocal Modulation of

Transcriptional Activities between HIV-1 Tat and MHC Class II Transactivator CIITA. *Biochem Biophys Res Commun*. 2000;279(2):494-499.

145. Accolla RS, Mazza S, Barbaro ADL, Maria AD, Tosi G. The HLA class II transcriptional activator blocks the function of HIV-1 Tat and inhibits viral replication. *Eur J Immunol*. 2002;32(10):2783-2791.
146. Mudhasani R, Fontes JD. Inhibition of class II trans-activator function by HIV-1 tat in mouse cells is independent of competition for binding to cyclin T1. *Mol Immunol*. 2002;38(7):539-546.
147. Lochhead RB, Ordoñez D, Arvikar SL, et al. Interferon-gamma production in Lyme arthritis synovial tissue promotes differentiation of fibroblast-like synoviocytes into immune effector cells. *Cell Microbiol*. 2018;21(2):e12992.
148. Mulder DJ, Pooni A, Mak N, Hurlbut DJ, Basta S, Justinich CJ. Antigen presentation and MHC class II expression by human esophageal epithelial cells: role in eosinophilic esophagitis. *Am J Pathol*. 2011;178(2):744-753.
149. Arancibia-Cárcamo CV, Osawa H, Arnett HA, et al. A CIITA-independent pathway that promotes expression of endogenous rather than exogenous peptides in immune-privileged sites. *Eur J Immunol*. 2004;34(2):471-480.