Hypothesis

Selective Antigen Presentation by Major Histocompatibility Complex Class I and II Molecules: A Hypothesis

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Selective Antigen Presentation by Major Histocompatibility Complex Class I and II Molecules: A Hypothesis

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Abstract

The major histocompatibility complex (MHC) class I and II molecules (abbreviated as MCH-I and MHC-II) are specialized in antigen presentation. Unlike the T cell receptors (TCRs), which have great variability, the MHC-I and MHC-II molecules have very limited variations. It is apparent that the MHC-I and MHC-II molecules per se do not have the built-in ability to distinguish the huge population of self-peptides from antigenic nonself-peptides. At present, the precise mechanism for the selective presentation of antigenic peptides by both MHC-I and MHC-II molecules is unclear. For an MHC-II molecule to gain the ability to selectively present antigenic (mostly foreign) peptides, it is hypothesized herein that all naïve $CD4^+$ T cells in the body will release extracellular vesicles (EVs) which are specially designed for uptake by the antigen-presenting cells; these EVs contain mRNAs that will be translated into an intracellular version of the TCR proteins ($iTCR^{II}$), which will help select antigenic peptides for presentation by the MHC-II molecules. Similarly, it is hypothesized that the activated $CD4^+$ T cells will also release EVs which contain mRNAs for another intracellular version of the TCR proteins ($iTCR^{I}$) which will help the infected somatic cells to select the antigenic peptides (mostly from invading pathogens) for presentation by the MCH-I molecules. Understandably, while the $iTCR^{II}$ proteins will work closely with MHC-II molecules in the exogenous endocytic pathway, the $iTCR^{I}$ proteins will work closely with MHC-I molecules in the endogenous pathway. In this paper, a few other related hypotheses are also proposed, which jointly offer a feasible cellular mechanism for the selective presentation of antigenic peptides by both MHC-I and MHC-II molecules. While the proposed hypotheses are supported by some experimental observations, it is hoped that these hypotheses will stimulate future experimental testing and enhance our understanding of the complex process of antigen presentation.

Keywords: antigen presentation; major histocompatibility complex molecules; T cell receptor; antigen-presenting cells; dendritic cells; EV; extracellular vesicles

1. Introduction

While the functions of B cells and antibodies are mostly for ridding the body of soluble/free toxins, viruses and bacteria [1], the functions of T cells are for monitoring the status of host cells for signs of viral infection and malignant transformation [2,3]. This difference in the functions of B and T cells is also reflected in how B and T cells recognize their respective antigens. While antibodies can directly bind to antigens in solution or present on the surface of pathogens [1], the majority of T cells are specialized in recognizing antigens only after they have been processed by the host cell and presented, in the form of short peptides, in complex with a plasma membrane-bound major histocompatibility complex (MHC) class I or class II (MHC-I or MHC-II) molecule [4–8].

MHC-II molecules are expressed primarily on cells of the immune system, and particularly by dendritic cells (DCs), macrophages, B cells, and thymic cortical epithelial cells [9–11]. Many somatic cells can be induced to express MHC-II molecules under certain conditions, such as during sustained

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inflammation and under interferon-γ (INF-γ) stimulation [11]. In contrast, MHC-I molecules are expressed by most nucleated somatic cells, and their presence are crucial for cytolysis of infected somatic cells by the $CD8^+$ cytotoxic T cells. These $CD8^+$ T cells kill cells on which they recognize the antigen and help eliminate cells infected with viruses or bacteria that live in their cytosol. In addition, $CD8^+$ T cells also recognize allogeneic MHC-I expressed on organ transplants and cause their rejection or recognize mutated self-proteins on cancer cells and help eliminate them [6,12]. Both naïve $CD4^+$ and $CD8^+$ T cells can become armed effector T cells after encountering their cognate antigen once it has been processed and presented by the activated DCs.

While several hundred different allelic variants of MHC-I and MHC-II molecules have been identified in humans, any one individual only expresses a very small subset of these molecules — up to 6 different MHC-I molecules and about 12 different MHC-II molecules [9,13–15]. Unlike immunoglobulins (Ig), B cell receptors (BCRs) and TCRs, which all have great variability, the MHC-I and MHC-II molecules have very limited variations. It is apparent that the MHC-I and MHC-II molecules per se do not have the built-in ability to distinguish a huge population of self-peptides from foreign antigenic peptides. Structural analysis showed that the MHC-I molecules can hold different peptides which have the right size (8–10 amino acids in length, 9 preferred) and meet the general structural requirements during their presentation to cytotoxic $CD8^+$ T cells [16–18]. Similarly, MHC-II molecules in APCs can hold antigenic peptides with a suitable size (13–18 amino acids in length) during their presentation to naïve $CD4^+$ T cells [11,16]. At present, the mechanisms by which the MHC-II and MHC-I molecules selectively present antigenic nonself peptides are still unclear. In this paper, a number of related hypotheses are proposed, which jointly offer a feasible mechanistic explanation for the selective presentation of antigenic nonself peptides by both MHC-II and MHC-I molecules. The proposed hypotheses are briefly discussed below, along with a discussion of the supporting evidence.

2. Hypotheses and Supporting Evidence

2.1. Many APCs Ectopically Express an Intracellular Version of the TCR Proteins Originally Created in Naïve $CD4^+$ T Cells

As mentioned above, the MHC-II molecules in an APC can hold different peptides with the right size and suitable general structure, and as such, these proteins do not have the built-in ability to distinguish self-peptides from foreign antigenic peptides. To gain the ability to selectively present only antigenic (mostly foreign) peptides, it is hypothesized that all naïve $CD4^+$ T cells in the body will release extracellular vesicles (EVs) which are specially designed for APCs, i.e., these EVs will be selectively taken up by the APCs. The naïve $CD4^+$ T cell-derived EVs contain mRNAs that can be translated into an intracellular version of the TCR proteins, and these intracellular TCRs have the same antigen-binding site (binding pocket) as the regular TCRs present on the surface of a naïve $CD4^+$ T cell which produces and releases the EVs. Since one naïve $CD4^+$ T cell only produces one type of TCR, the EVs released from a naïve $CD4^+$ T cell will also contain the mRNAs for a single type of intracellular TCR proteins. It should be noted that the naïve $CD4^+$ T cell-produced mRNAs can only be translated into an intracellular version of the TCR proteins which will be present in the APCs in selected subcellular compartments (but not on their cell surface). It is further hypothesized that these intracellular TCRs will work in concert with the MHC-II molecules (most likely in the endosomes of the endocytic pathway) [19], and their function is for the selection of antigenic (mostly foreign) peptides for presentation by the APC's MHC-II molecules. Note that these intracellular TCRs are not for antigen presentation by MHC-I molecules; a slightly different mechanism is used for MHC-I molecules (discussed later).

To help distinguish from a regular TCR protein which is present on the surface of a T cell, this intracellular version of the TCR that is ectopically expressed in an APC is referred to as **$iTCR^{II}$**; here, "**i**" denotes its intracellular localization, and the superscript "**II**" denotes its functional coupling with the MHC-II molecule for the selective presentation of antigenic peptides.

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Since a naïve $CD4^+$ T cell only expresses one type of TCRs and since these TCRs have already gone through the selection process during thymocyte maturation in the thymus, theoretically the TCR can only bind antigenic (mostly foreign) peptides but not non-antigenic self-peptides. Because the peptide-binding site structure (and its amino acid sequence) of the $iTCR^{II}$ protein translated in an APC is the same as the regular TCR present on the surface of a corresponding $CD4^+$ T cell, it is understood that the $iTCR^{II}$ in an APC normally will not bind self-peptides. Because of this property, it is believed that most non-antigenic self-peptides normally are not presented on the surface of an APC in complex with MHC-II (more discussion on this subject is provided later). This proposed mechanism would help explain why APCs in the body can selectively present only the antigenic peptides derived from engulfed pathogens.

Since there are so many different naïve $CD4^+$ T cells circulating in the body and each one of them carries a different TCR (with a different binding site structure), it is speculated that an APC usually can receive EVs (which contain mRANs for $iTCR^{II}$s) from many different naïve $CD4^+$ T cells. In other words, it is speculated that the $iTCR^{II}$ mRNAs from different naïve $CD4^+$ T cells may be concomitantly translated into different $iTCR^{II}$ proteins inside a naïve APC as a way to increase efficiency. An alternative possibility is that one APC may only be permitted to take up one type of $iTCR^{II}$ mRNAs — in a way which is somewhat similar to the phenomenon seen in an egg cell which normally only permits the entrance of one sperm. However, this alternative possibility is believed to be far less likely to happen during the initial presentation of an antigen to a naïve $CD4^+$ T cell by an APC.

It is known that during antigen processing, different antigenic peptides may come from different subcellular compartments. These compartments, which are usually separated by cellular membranes, include the cytosol and various vesicular compartments involved in endocytosis and secretion. Pathogenic bacteria that proliferate outside the cells can be taken up, along with their toxic products, by phagocytosis, receptor-mediated endocytosis, or macropinocytosis into endosomes and lysosomes. Similarly, virus particles and parasite antigens in extracellular fluids can also be taken up by these routes and degraded. In professional APCs, their early endocytosomes contain protein-degrading enzymes that can preferentially catalyze the formation of 13–18 amino acid-long peptides. It is speculated that the $iTCR^{II}$s likely is in close association with the early endocytosomes, and their presence will help select antigenic (mostly foreign) peptides that are being trimmed to 13–18 amino acids in length inside the early endocytosomes. The antigenic peptides that are bound by the $iTCR^{II}$s will be selectively transported to the immunoproteosomes for packaging with the MHC-II molecules, and the peptide–MHC-II complex will then be presented on the cell surface [5,11,20]. As such, only the $iTCR^{II}$-bound peptides will be selected for presentation by an APC's MHC-II molecules.

According to the proposed hypothesis, it is understood that most APCs have the ability to selectively uptake EVs released by naïve $CD4^+$ T cells; otherwise, these APCs cannot selectively present antigenic peptides in complex with their MHC-II molecules. While DCs, macrophages and B cells are often referred as professional APCs, some of the somatic cells in the body which usually do not have antigen-presenting function may transiently gain the function to present foreign antigens under certain pathologic conditions, usually during sustained inflammation and under INF-γ stimulation. It is speculated that when certain populations of somatic cells are preferentially infected by the invading pathogens, they may transiently gain the ability to present antigens like an APC such that it will increase the efficiency of selectively presenting antigens derived from the invading pathogens. Under such conditions, one of the important steps that will arm these regular somatic cells to be APC-like cells is that these somatic cells need to gain the ability to selectively uptake the EVs produced by naïve $CD4^+$ T cells for the expression of the $iTCR^{II}$s. The presence of the $iTCR^{II}$ proteins will then enable them to selectively present antigenic peptides from the invading pathogens. In addition, these nonprofessional APCs will also be induced to express MHC-II molecules and costimulatory signals. After these steps, they are properly deputized for antigen presentation, usually for a given duration under certain situations.

Among the various professional APCs, it is known that there are marked differences in the levels of MHC-II expression. In some cases, MHC-II expression depends on the levels of their activation.

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APC activation usually occurs following their interaction with pathogens (containing pathogen-associated molecular patterns, PAMPs) by the pattern recognition receptors (PRRs) coupled with cytokine signaling, which jointly alters gene expression in these APCs, including significant increases in the expression of MHC-II molecules.

B cells constitutively express MHC-II molecules at low levels, and possess antigen-specific surface receptors [11]. Theoretically, B cells are excellent professional APCs, as these cells can recognize and selectively concentrate the invading pathogens based on the specific BCRs present on the surface of these cells. It is speculated that B cells (like other professional APCs) also express the iTCR^{II}s (through receiving the EVs released from CD4$^+$ naïve T cells). Assume that a B cell internalizes extracellular protein particles from a pathogen through its BCR-mediated endocytosis, and the internalized proteins contain the antigenic peptide that has a high affinity for the iTCR^{II}s and then selected for presentation on its surface in complex with MHC-II molecules. These B cells will present the antigenic peptide to a naïve CD4$^+$ T cell for its activation. The activated CD4$^+$ T cell will subsequently be differentiated into a special clone of helper T cells, which can selectively activate this clone of B cells, leading to its proliferation and clonal expansion. Therefore, more B cells with the same ability to recognize the invading pathogens will be present in the circulation to capture the pathogens (or their proteins) for antigen processing and presentation. In addition, the expansion of this B cell clone will also increase the chances to receive EVs from other naïve CD4$^+$ T cells, and some of the naïve T cells may express iTCR^{II} proteins which have even higher binding affinity than the iTCR^{II} protein ectopically expressed in the initial B cell which had led to its activation and expansion. Lastly, the specific antibodies produced and released by this clone of B cells (i.e., plasma cells) will also help enhance the phagocytosis of the invading pathogens by other professional DCs or enhance their clearance by macrophages [1]. Notably, when the DCs capture these "antibody-tagged" pathogens, many of these DCs have the ability to selectively present the pathogen-derived peptides as they likely have already expressed the iTCR^{II} proteins.

Lastly, it should be noted that CD4$^+$ T cells are highly capable of produce and release exosomes to regulate the body's immune system functions [21–23]. Additionally, earlier studies have reported that there is an ectopic expression of TCR-like immune receptor in non-T cells in humans, such as neutrophils and macrophages [24–29]. Interestingly, the ectopic expression of TCR-like immune receptors in neutrophils in humans was found to be present across the entire life span, although the repertoire diversity declines in old age [26]. Additionally, the TCR $\alpha\beta$ were detected in macrophages in atherosclerosis lesions [26] and tumor microenvironments [27]. Similarly, TCR $\alpha\beta$ bearing macrophages accumulate at the inner host-pathogen contact zone of caseous granulomas from patients with lung tuberculosis [25]. Based on all these observations, it is quite clear that the ectopic expression of TCR $\alpha\beta$ is associated with certain functions. It is speculated that these TCR-like proteins likely are the intracellular TCR proteins as hypothesized in this paper, and their mRNAs are produced by CD4$^+$ T cells.

2.2. An Activated Naïve CD4$^+$ T Cell Has Multiple Fates

The engagement of a naïve CD4$^+$ T cell with an APC usually takes place in the T-cell zone of a secondary lymphoid tissue. It is known that the binding of the TCR of a naïve CD4$^+$ T cell with the MHC-II–peptide complex on an APC, even aided with the binding of CD4 (jointly referred to as "Signal-1"), is not sufficient to fully activate a naïve T cell [30]. For the full activation to occur, the CD28 coreceptor on a CD4$^+$ naïve T cell must also engage its ligand (CD80 or CD86) on the APC (which forms "Signal-2") [30]. It is believed that the requirement of CD28 to engage its ligand on a professional APC serves as a safeguard which helps a naïve CD4$^+$ T cell to reduce the likelihood that the peptide complexed to MHC-II of an APC is from the self-components. It is known that CD80 and CD86 (both are ligands of CD28) are present only on professional APCs such as DCs, macrophages, and activated B cells. Moreover, the levels of their expression are selectively increased following antigen uptake mediated by APC's innate immune receptors (such as BCRs and PRRs). As such, the CD4+ naïve T cells, through specific interaction between its CD28 and APC's CD80/CD86, will be

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assured that the peptide-presenting APC had just engulfed foreign antigens. On the other hand, if a peptide-presenting APC does not express high levels of CD80 or CD86, then a $CD4^+$ naïve T cell will be alerted that the APC might be accidentally presenting a peptide of the host cells. Lastly, an APC following the activation of its innate immune receptors will also be induced to secrete cytokines. Different cytokine mixtures are secreted depending on the types of APCs, the identities of the PRRs being activated, and the cytokine environments the APCs are in. The cytokines secreted by an APC (which forms "Signal-3") are needed to complete the initial activation of a naïve $CD4^+$ T cell [30].

Here, it is further hypothesized that following the initial activation of a naïve $CD4^+$ T cell by an APC (usually in the T cell zone of a secondary lymphoid tissue), this $CD4^+$ T cell will need to travel back to the medulla region of the thymus to undergo another round of negative selection. This step serves as a double check to ensure that this activated CD4+ T cell does not, by any chance, react strongly with any of the self-peptides presented by thymic medullar cells. If the activated $CD4^+$ T cell does not bind to any of the self-peptides in the thymus with a meaningful binding avidity, which means that it has passed the double check, then this activated $CD4^+$ T cell is licensed to undergo proliferation inside the thymus to become a small clone of $CD4^+$ effector T cells. As listed below, these $CD4^+$ T cells will then further differentiate into different subsets of immune cells with diverse functions to combat the invading pathogens.

- A subset of the fully-activated $CD4^+$ T cells will become a unique group of effector/helper T cells which will release EVs specially designed to arm infected somatic cells in the body for recognition by activated $CD8^+$ T cells (discussed in *Section 2.3*).
- A subset of the activated $CD4^+$ T cells will become many other types of helper T cells (discussed in *Section 2.4*).
- While still inside the thymus, if a fully-activated $CD4^+$ T cell happens to cross-react with self-peptides presented on mTECs (in complex with the MHC-II molecule), then this self-reactive $CD4^+$ T cell will be differentiated into a clone of regulatory T (T_{REG}) cells, which will help suppress autoimmunity in the body (discussed in *Section 2.5*). On the other hand, if a $CD4^+$ T cell is only partially activated by an APC, *i.e.*, in the presence of Signal-1 but in the absence of Signal-2, it would mean that the antigenic peptide presented by the APC may not come from an invading pathogen. There are two outcomes: If the partially-activated $CD4^+$ T cell is self-reactive, then it will be differentiated into a subset of $CD4^+$ regulatory T cells; however, if the partially-activated $CD4^+$ T cell has no self-reactivity, then it may help regulate anticancer immunity (discussed in *Section 2.5*) or may go into anergy.

2.3. A Subset of the Activated Effector $CD4^+$ T Cells Will Release EVs Specially Designed to Arm Pathogen-Invaded Somatic Cells for Antigen Presentation

After a fully-activated $CD4^+$ T cell has successfully undergone another round of negative selection in the thymus, it will return to the T-cell zone of a secondary lymphoid tissue. The transcription of genes for both IL-2 and the α chain (CD25) of the high-affinity IL-2 receptor will be upregulated in this activated T cell. This cytokine, along with other factors, will act on the activated $CD4^+$ T cell, inducing robust cell division. This clone of activated $CD4^+$ T cells will then be differentiated into different subsets of effector/helper T cells, with each subset playing a specific role in the concerted immune response against the invading pathogens.

Specifically, it is hypothesized that a subset of the activated effector/helper $CD4^+$ T cells will start to release large amounts of EVs which specifically target recently-infected somatic cells in the body, and these EVs contain mRNAs which can be translated into another intracellular version of the same TCR. Note that this version of the intracellular TCR will be expressed in these recently-infected somatic cells (which will also start to express the MHC-I molecules), and its function is to work jointly with the MHC-I molecule for the selection of antigenic peptides for presentation by MHC-I. For convenience, this intracellular version of the TCR protein is referred to as **$iTCR^I$** ("**i**" denotes its intracellular localization, and the superscript "**I**" denotes its functional coupling with the MHC-I

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molecule). Understandably, the iTCR^{I} molecules will be closely associated with cellular organelles of the internal protein metabolic pathway, in a similar manner as the MCH-I proteins.

Since a pathogen may lead to the activation of many different CD4^{+} T cells, it is predicted that a somatic cell may uptake EVs from different clones of activated CD4^{+} T cells. As a result, multiple versions of the iTCR^{I} proteins may be concomitantly present and translated in one somatic cell, which will facilitate the presentation of multiple antigenic peptides at the same time. In so doing, it will help increase the efficiency of antigen presentation by infected somatic cells.

Understandably, the activated CD4^{+} T cell-derived mRNAs which are ectopically present in MCH-I-expressing somatic cells will be slightly different from the naïve CD4^{+} T cell-derived mRNAs which are present in APCs, as these two mRNAs will be translated into intracellular TCR proteins (*i.e.*, iTCR^{I} and iTCR^{II}) with different subcellular localizations—despite the fact that they share the same peptide-binding site structure. While iTCR^{I}s in infected somatic cells are destined for their joint functioning with MHC-I molecules, iTCR^{II}s in APCs are functionally coupled with MHC-II molecules in a different subcellular compartment. It is speculated that the iTCR^{I} and iTCR^{II} proteins contain specific peptide sequences in their structures which will selectively guide them to the right subcellular locations to perform their specialized functions.

Here, it is worth noting that the total numbers of different iTCRIIs present in all APCs in the body are enormous, essentially the same as the total numbers of all different types of TCRs produced by all naïve CD4^{+} T cells. By contrast, the total numbers of different TCRs present in somatic cells are usually far smaller, as only those TCRs of fully-activated CD4^{+} T cells (which have high binding affinity for the pathogens' antigenic peptides) are selected.

After the pathogen-invaded somatic cells receive EVs released from the activated CD4^{+} T cells and start to express the intracellular iTCR^{I} proteins, these somatic cells will be equipped with the ability to selectively present the antigenic peptides on their cell surface in complex with the MHC-I molecules, and are ready for selective targeting by the activated cytotoxic CD8^{+} T cells.

Notably, not all somatic cells will automatically have the ability to uptake EVs released by activated CD4^{+} T cells. It is speculated that only those "activated" somatic cells will upregulate the expression of MHC-I molecules and other required costimulatory proteins (such as CD80/CD86), along with the proteins specifically required for the selective uptake of EVs released by activated CD4^{+} T cells. It is highly possible that activation of nucleated somatic cells may be triggered by the presence of the invading pathogens inside these cells, which will then start to release relevant cytokines for their own activation as well as for the activation of the neighboring cells.

Based on the above-proposed mechanism, it is understood that because the intracellular iTCR^{I}s expressed in many somatic cells have already gone through rounds of stringent selection to avoid self-antigens, so they have very little chance of binding (with high affinity) to host's own cellular peptides for MHC-I presentation. Here, it should be further noted that this selective presentation of only antigenic (mostly foreign) peptides by MHC-I molecules does not necessarily mean that none of the self-peptides will be presented by MHC-I on the cell surface of a normal cell. In fact, it has been reported that normal cells contain MHC-I proteins on their cell surface presenting some of the normal cellular peptides [31,32], and cancer cells can evade the immune system through the selective loss of antigen presentation by MHC-I, such that it may help avoid presenting their own cancer antigens [33]. It is hypothesized that some non-antigenic self-peptides (presumably very limited varieties) which are specially made in somatic cells may be presented intentionally on the surface of normal cells simply for the maintenance of the immune system functions. It is likely that these 'generic' self-peptides belong to a very small group of self-peptides that normally do not have any binding affinity for the TCRs. If this speculation is correct, then it is likely that there is a cellular protein (or a few proteins) which is functionally very similar to the intracellular iTCR^{I}s, and these proteins can selectively bind to the generic, non-antigenic self-peptides and hand them over to MHC-I molecules for presentation at the surface of normal cells.

In summary, it is hypothesized that the fully-activated T cells will release EVs which contain specific mRNAs and will be selectively taken up by infected somatic cells in the body for translation

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into iTCR^{I} proteins. In so doing, it will prepare the infected somatic cells to selectively express iTCR^{I}s such that they can selectively present the invading pathogens' antigenic peptides on their cell surface in complex with MHC-I molecules to prepare for attacks by cytotoxic CD8^{+} T cells. Although it is known that there are many different subtypes of CD4^{+} effector/helper T cells in the body, it is presently unclear which subset(s) of the CD4^{+} effector/helper T cells actually fulfill this function of producing EVs to selectively arm pathogen-invaded somatic cells.

2.4. A Subset of Activated CD4^{+} T Cells Will Become Other Helper T Cells

The presentation of a pathogen-derived antigenic peptide by an APC, irrespective of where the antigenic proteins come from (*i.e.*, either from a virus, bacterium, worm or parasite, or others), all utilizes the same mechanism, *i.e.*, the antigenic peptide from a pathogen is presented to a naïve CD4^{+} T cell by an APC in complex with its MHC-II molecules. After that, the activated CD4^{+} naïve T cell will return to the thymus to ensure that it does not cross-react with any self-peptides, and then a fraction of the activated CD4^{+} T cell will leave the thymus and become clones of effector/helper T cells of different subtypes/subsets. As discussed above, some of the activated CD4^{+} T cells will start to release EVs (which contain mRNAs for iTCR^{I}) to arm infected somatic cells with the ability to selectively present pathogen-derived antigenic peptides. As discussed below, there are other subsets of CD4^{+} effector/helper T cells, which will work, in concert, in the fight against the invading pathogens.

Because different invading pathogens survive in different cellular environments (some survive inside the cells, whereas others survive outside the cells), different types of immune responses are selectively activated to deal with each type of the pathogens. Understandably, in order for the body to mount an effective immune response against a certain type of the invading pathogens, different subsets of the helper T cells are selectively formed depending on the types of the invading pathogens and also on the types of immune responses needed. Here, the question is: How does an activated CD4^{+} T cell know all these beforehand so that it can undergo the selective differentiation to form the right subset(s) of effector/helper T cells to precisely aid the immune responses against the invading pathogens? It is speculated that a naïve CD4^{+} T cell is effectively informed by the APC at the time of antigen presentation about the type of pathogens from which the antigenic peptide is derived and the exact type of APC which is presenting the antigen to the naïve CD4^{+} T cell. As we know, an APC will release "polarizing cytokines" (which constitute Signal-3), and these cytokines, along with Signal-1 and 2, will selectively alter the gene expression patterns of a CD4^{+} T cell being activated. Hence, the unique combination of Signal-1, 2 and 3 will dictate the future fate of this clone of T cells, *i.e.*, which subset(s) of the effector/helper T cells they will develop into. During this process, the neighboring innate immune cells will also aid in informing the naïve CD4^{+} T cell about the types of the invading pathogens through the unique cytokines they release.

Here, let us use B cells as an example to illustrate in more detail the proposed mechanism as to how a helper T cell can selectively activate the function of a specific B cell (or B cell clone). Assume that there is a specific B cell (or B cell clone) in the body which is labeled as ^{1}B cell. This ^{1}B cell serves as a professional APC and has just completed presenting the antigen (from an invading pathogen) to a CD4^{+} naïve T cell (labeled as ^{1}T cell). Here, it should be noted that because the naïve ^{1}T cell is selectively activated by the ^{1}B cell, it means that the ^{1}B cell contains the intracellular iTCR^{II} proteins which are also present on the surface of the ^{1}T cell; otherwise, the 1T cell would not be able to selectively recognize the antigenic peptide presented by the ^{1}B cell in complex with its MHC-II. During the antigen presentation by ^{1}B cell to naïve ^{1}T cell, cytokines are released from ^{1}B cell for the activation of ^{1}T cell. These polarizing cytokines (Signal-3), along with the binding of its TCR to an antigenic peptide (Signal-1) and the engagement of costimulatory ligands present on ^{1}B and ^{1}T cells (Signal-2), will lead to selective ^{1}T cell activation and induce a signature change in its gene expression which will determine its highly-specified fate. During the process, this ^{1}T cell is informed of which type of professional APCs has presented the antigenic peptide to it, and which family the pathogen belongs to. Assume that the fully-activated CD4^{+} ^{1}T cell now has successfully gone through the

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double checking in the thymus and is becoming a subset of effector/helper T cells, these cells can precisely recognize and thus help the ^{1}B cell (or its clone) but likely not other professional APCs. Since the B cells have so many different clones, here another question arises: how does the helper ^{1}T cell (or its clone) knows to selectively activate the ^{1}B cell (or its clone)? The explanation is rather straightforward based on the proposed mechanism: as the intracellular iTCR^{II}s in the original ^{1}B cell have the same peptide-binding site as the TCRs of the helper ^{1}T cell, naturally the ^{1}T cell can selectively recognize the ^{1}B cell through the antigenic peptide presented by the ^{1}B cell (in complex with its MHC-II molecule). Here, it is interesting to note that when the CD4^{+} ^{1}T cell encounters the ^{1}B cell for the first time and the TCR of the ^{1}T cell identifies the peptide presented by the MHC-II molecule of the ^{1}B cell, this first encounter serves the function of selective antigen presentation from the ^{1}B cell to the naïve CD4^{+} ^{1}T cell. However, when the activated ^{1}T cell becomes a helper ^{1}T cell, the second encounter between the helper ^{1}T cell and ^{1}B cell serves the function of selective activation of the ^{1}B cell (or its clone). Following this activation, the ^{1}B cell will undergo cell division to increase its numbers, and this ^{1}B cell clone will then perform a number of immunological functions in the fight against the invading pathogens. Therefore, it is understood that within the same subgroup of helper T cells (*e.g.*, T$_{H}$1 cells, which have numerous T$_{H}$1 clones), each clone of the helper T$_{H}$1 cells (such as the ^{1}T clone) will only selectively activate a specific clone of the other immune cells (such as the ^{1}B cells); these two clones of cells are selectively coupled because they share the same TCR structure and can bind the same antigenic peptide. Although the initial naïve CD4^{+} ^{1}T cell in this case is activated by ^{1}B cell, it is important to note that the activated ^{1}T helper cells may also activate other APCs that contain the same iTCR^{II}, besides the ^{1}B cell (or its clone).

Additionally, the helper ^{1}T cells may have other subsets, and they will jointly regulate the complex functions of body's immune system. For instance, different B cells can be activated to produce different antibodies (*e.g.*, IgG and Ig E), and they have different functions in defending pathogens of different groups (some live inside the host cells, and some live outside the host cells). It is likely that different helper T cell subsets are involved in regulating B cells to produce different Igs. The T$_{H}$1 and T$_{H}$17 subsets are thought to promote B cells to produce antibodies that contribute to cell-mediated immunity (*e.g.*, isotypes like IgG2a that can "arm" NK cells for cytotoxicity), whereas T$_{H}$2 cells promote B cells to produce antibodies that mediate the clearance of extracellular pathogens (*e.g.*, isotypes like IgE that induce the release of molecules that harm extracellular parasites).

2.5. Mechanism of the Formation of CD4^{+} Regulatory T Cells

The CD4^{+} regulatory T cells (T$_{REG}$) was first described in the late 1960s [34]. These T$_{REG}$ cells express FoxP3, a lineage-defining transcription factor [11,35–37], along with some other signature genes, such as CTLA-4 and high levels of the IL-2R α chain (CD25) [37,38]. Functionally, these T$_{REG}$ cells play a crucial role in immune suppression, ensuring tolerance to self-antigens [39,40], innocuous allergens [41,42] and commensal microflora [43].

How are the T$_{REG}$ cells formed in the body? In the case of CD4^{+} T$_{REG}$ cells, it is generally thought that a fraction of the thymocytes that experience high-affinity TCR interactions do not die by negative selection but instead develop into T$_{REG}$ cells. A number of mechanisms have been suggested in the past to help explain why these T cells, whose TCRs display high affinity for self-antigen in the thymus, are not eliminated by negative selection [44]. The best-known model is the "hit and run" hypothesis, which suggests that short, high-affinity but transient engagement of a T cell's TCR with the MHC–antigen complex in the thymic medulla favors the generation of T$_{REG}$ cells; however, more sustained, high-affinity TCR engagement in the thymus favors deletion of the self-reactive lymphocytes (via elimination).

Based on the new hypothesis developed here, a different possibility is entertained. Using the CD4^{+} T$_{REG}$ as an example, there are a few different scenarios which may lead to their formation, as briefly discussed below:

Scenario 1. A fully-activated but self-reactive CD4^{+} T cell will be converted to T$_{REG}$ cells. After a naïve CD4^{+} T cell is fully activated by an APC and then returns to the thymus to undergo another

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round of negative selection, if it happens that the TCRs of the activated $CD4^+$ T cell cross-reacts with a self-antigen presented by the thymic cell (mostly mTEC) in complex with its MHC-II molecules, then this $CD4^+$ T cell will be converted to a T_{REG} cell, which will then undergo proliferation (in the thymus initially and later in secondary lymphoid tissues) to form a T_{REG} clone specific for this self-antigen. Here, there are two possibilities with the TCR of this T cell: one is that its TCR indeed can recognize the invading pathogen's peptide, but it also cross-reacts with a self-antigen. The other possibility is that its TCR actually cannot recognize the pathogen's peptide but just happens to cross-react with a self-antigen. In either case, it would be detrimental to the host if this $CD4^+$ T cell is allowed to develop into mature $CD4^+$ effector T cells and cytotoxic $CD8^+$ T cells. Instead, this clone of self-reactive $CD4^+$ T cells will be converted into a clone of T_{REG} cells (and memory T_{REG} cells), and understandably, their function is to suppress autoimmunity against this particular self-antigen in the peripheral tissues. This subset of T_{REG} cells may be somewhat similar in function to the so-called tissue-resident thymic T_{REG} cells (tT_{REG} cells).

In the literature, there is another subset of T_{REG} cells, called peripheral T_{REG} cells (pT_{REG} cells). It has been suggested that the pT_{REG} cells may arise from $CD4^+$ T cells that are activated in secondary lymphoid tissues after exposed to antigen in the context of TGF-β and IL-10 cytokines [45]. However, in view of the proposed hypothesis, it is less likely that the pT_{REG} cells arise entirely in the periphery; it is more likely that these pT_{REG} cells also need to return to the thymus during their development to confirm their cross-reactivity against self-antigens presented by the mTECs and then are commissioned to be pT_{REG} cells. In other words, there might be no real pT_{REG} cells that are generated entirely in the periphery without the need to return to the thymus.

Mechanistically, the $CD4^+$ T_{REG} cells may achieve the suppression of the immune response against a specific self-antigen via a variety of means, as summarized below:

i. The T_{REG} cells may modulate the functions of other immune cells. As aforementioned, Signal-2 is produced by the costimulatory ligands expressed on APCs which then interact with the costimulatory receptors (CD28 or ICOS) expressed on T cells [46]. However, co-inhibitory receptors, such as CTLA-4, PD-1 and BTLA expressed on T_{REG} cells, can bind to their respective ligands on APCs [47,48]. Since these APCs express the $iTCR^{II}$ protein which is cognate to the TCRs on the $CD4^+$ T_{REG} cell and can selectively present the same self-reactive antigen, the interaction of the $CD4^+$ T_{REG} with the APCs will result in their inactivation. It is hypothesized that the inactivated APCs will then go on to help inactivate all other T cells which can recognize this particular self-reactive antigen presented by the APCs. Stated differently, those APCs that are inactivated by T_{REG}s will be commissioned to selectively inactivate both the naïve $CD4^+$ T cells and $CD8^+$ CTL precursors whose TCRs can recognize this self-antigen. The inactivated $CD8^+$ CTL precursors will become anergic [49]. Offering partial support for this suggestion, earlier studies have shown that the T_{REG} cells can effectively reduce the expression of various costimulatory molecules on APCs, but not MHC-I or MHC-II [46]. Notably, it is also known that T_{REG}-mediated immune suppression is highly antigen specific. This is readily understood in the light of the proposed hypothesis as the T_{REG} cells will only inactivate those APCs that selectively express the cognate $iTCR^{II}$ protein and thus can present the same self-reactive antigen.

ii. The $CD4^+$ T_{REG} cells have been shown to kill immune cells (such as $CD8^+$ effector T cells) directly, by means of granzyme and perforin [50]. For the $CD4^+$ T_{REG} cells to selectively kill $CD8^+$ effector T cells, it is hypothesized that these T_{REG} cells need to jointly express the $iTCR^I$ protein and MHC-I molecules such that they can acquire the ability to present the antigenic peptide through their MHC-I molecules. In this way, the T_{REG} cells can directly interact with the effector $CD8^+$ T cells, and the tight-binding binding interactions between a $CD4^+$ T_{REG} cell and an effector $CD8^+$ T cell will result in the inactivation or direct killing of the effector $CD8^+$ T cell.

iii. Many studies have shown that T_{REG} cells can also suppress the immune system functions in a rather nonspecific manner [51]. For instance, T_{REG} cells can secrete inhibitory cytokines (*e.g.*, IL-10, TGF-β and IL-35), suppressing the activity of other nearby T cells and APCs. Additionally, as T_{REG} cells express high levels of CD25 (the high-affinity IL-2 receptor), they can act as a sponge, absorbing

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this growth- and survival-promoting cytokine and further discouraging expansion of local immune-stimulatory effector T cells [51].

Scenario 2. A partially-activated but self-reactive CD4+ T cell will also become TREG cells. If a naïve CD4+ T cell's TCR is engaged and partially activated by an APC, *i.e.*, in the presence of Signal-1 but in the absence of Signal-2 (the costimulatory signal CD80/CD86), the situation would indicate to the T cell that the antigenic peptide presented by an APC in complex with its MHC-II may not come from an invading pathogen. During PRR-mediated engulfment of an invading pathogen by a professional APC, PRR activation will lead to enhanced antigen-presenting activity through up-regulation of MHC-II molecules and costimulatory ligands (CD80/CD86). As such, when the costimulatory ligands (CD80/CD86) are absent in the APCs, it is quite possible that the peptide being presented by an APC may not be derived from an invading pathogen.

Therefore, it is hypothesized that when a CD4+ naïve T cell is partially activated in the absence of costimulatory ligands, this CD4+ naïve T cell will also return to the thymus to go through another round of negative selection to determine whether this T cell can react with any self-peptides. If it does, then it would mean that this CD4+ naïve T cell indeed expresses a self-reactive TCR — this T cell might have escaped the negative selection and left the thymus during its initial maturation/selection process. It is speculated that this self-reactive CD4+ T cell will be activated to become a CD4+ TREG clone in the thymus, and after leaving the thymus, these TREG cells will serve to suppress the future activation of auto-reactive naïve CD4+ T cells and the CD8+ CTL precursors that express the same TCR and can recognize this self-peptide. The mechanisms of immune suppression are essentially the same as described above in *Scenario 1*.

Scenario 3. A partially-activated CD4+ T cell with no self-reactivity may help regulate anticancer immunity. If a partially-activated CD4+ T cell does not cross-react with any of the self-peptides presented by thymic cells, it implies that this antigen likely is from transformed or cancer cells. Should this be the case, then this CD4+ T cell after leaving the thymus may be presented with a target peptide (in complex with MHC-II) and gets activated peripherally again, then it will trigger the anticancer immune responses. Here, I will not further speculate on the subsequent processes, but just like to say that the anticancer immune responses may take much longer duration compared to the anti-pathogen immune responses as the cancer cells in the body, unlike the invading bacteria and viruses, usually develop rather slowly. It is understood that the body's immune system may take significantly longer time and a lot more steps to ascertain whether the antigenic peptide is really from the transformed cancer cells or from the body's normal cells. Understandably, during the development of anticancer immune response, urgency (*i.e.*, timeliness) would take a back seat in comparison with the anti-pathogen immune response; whereas accuracy is of paramount importance because if a mistake is accidentally made during the hasty process, it would have very severe, even life-threatening, consequences of inducing autoimmunity. It is, therefore, speculated that the immune responses against the transformed or cancer cells may be somewhat similar to the processes of "chronic rejection" against an organ transplant, which usually takes much longer time to slowly develop. The apparent slowness in these process is to perform multiple check to assure that the antigens are indeed not the self-antigens.

On the other hand, if a partially-activated CD4+ T cell which has no self-reactivity is not further presented with any antigens in the body for a certain period of time after it leaves the thymus, then it may become unresponsive (anergic). Based on what is reported in the literature, the anergic T cells usually do not secrete cytokines nor proliferate in response to subsequent stimulations, which is readily understood. It is speculated that this T cell will end up undergoing cell death after some time. Offering partial support for this suggestion, earlier experiments by Jenkins and colleagues showed that when CD4+ T-cell clones are stimulated in vitro through the TCR alone in the absence of costimulation, they become anergic [52].

In summary, when the TCRs of a fully or activated CD4+ T cell can cross-react with self-peptide(s) while back in the thymus, this T cell will be converted to CD4+ TREG cells, which will serve the function of selectively suppressing the autoimmune reactions against this class of self-antigens.

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Interestingly, for a partially-activated $CD4^+$ T cell with no self-reactivity, it poses the possibility that this antigen may come from transformed or cancer cells. It is speculated that if this $CD4^+$ T cell later indeed encounters cancer antigens again in the periphery, then it may help activate the anticancer immunity. However, if this $CD4^+$ T cell is not further activated by peripheral antigens, then it will become anergic, undergoing apoptosis.

3. Concluding Remarks

It is known that the MHC-I and MHC-II molecules do not have the built-in ability to distinguish self-peptides from foreign antigenic peptides, and the precise mechanism for their ability to selectively present antigenic (mostly foreign) peptides is not clear at present. Here, it is hypothesized that all naïve $CD4^+$ T cells will release EVs containing specific mRNAs for the selective uptake by APCs. These mRNAs will be translated into the intracellular $iTCR^{II}$ proteins which will help select nonself peptides for presentation by the MHC-II molecules. It is speculated that a naïve APC may receive EVs (which contain mRANs for different $iTCR^{II}$s) from many different naïve $CD4^+$ T cells, as a way to enhance efficiency. Following the full activation of a naïve $CD4^+$ T cell by an APC, it is hypothesized that this T cell will return to the thymus for another round of negative selection to make sure that it does not cross-react with self-components. After this T cell has successfully passed the double check, then it is licensed to undergo proliferation inside the thymus and in peripheral secondary lymph tissues to become a small clone of $CD4^+$ effector T cells. It is hypothesized that a subset of the $CD4^+$ effector T cells will release EVs which contain mRNAs encoding the $iTCR^I$ protein, and these EVs are specially designed for uptake by pathogen-infected somatic cells in the body. These $iTCR^I$ proteins will work closely with the MHC-I molecules to selectively present pathogen-derived antigenic peptides in infected somatic cells.

The proposed hypotheses are of value in offering a different angle in looking at the immensely complex process of selective antigen presentation. As discussed in the paper, there are some experimental (mostly indirect and circumstantial) observations which offer partial, tangible support for the proposed hypotheses. It is hoped that some of the proposed hypotheses will attract and stimulate future experimental testing of the relevant hypothetical elements proposed.

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Abbreviations

The following abbreviations are used in this manuscript:

MHC-I	major histocompatibility complex class-I molecule
MHC-II	major histocompatibility complex class-II molecule
TCR	T cell receptor
BCR	B cell receptor
$iTCR^I$	intracellular version of the TCR protein functionally coupled with MHC-I molecule
$iTCR^{II}$	intracellular version of the TCR protein functionally coupled with MHC-II molecule
DC	dendritic cell
APC	antigen-presenting cell
CTL	cytotoxic T cell
T_{REG} cell	regulatory T cell
EV	extracellular vesicle
INF	interferon
PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptor
mTECs	medullary thymic epithelial cells

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