The role of cholesterol in TCR signalling in autoimmune diseases

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Abstract
Cholesterol is an important structural and functional component of the plasma membrane. In this review, we focus on T cells and the role of cholesterol in T cell antigen receptor (TCR) signalling, which contributes to autoimmune diseases. Cholesterol binding to the TCR leads to an increased formation of TCR nanoclusters, increasing the avidity of T cells towards the antigen and enabling TCR cooperativity, thus increasing the sensitivity of the T cell. Further, cholesterol is important in the formation of certain lipid nanodomains, called lipid rafts that concentrate signalling molecules and thus also promote TCR signalling. T cell and lipid dysregulation play a key role in the pathogenesis of autoimmune diseases such as systemic lupus erythematosus (SLE). Typically, in SLE, hyper-responsive and exaggerated TCR signalling occurs, partially caused by cholesterol accumulation in the plasma membrane. This might lead to enhanced TCR nanoclustering and lipid raft formation. Thus, targeting lipid metabolism in T cells.
Introduction

T cells are an essential part of the adaptive immune system along with B cells. On one hand T cells function as cytotoxic cells to destroy pathogen-infected cells or tumour cells, and as helper cells to aid other immune cells. On the other hand T cells are also involved in the recognition of healthy cells in the case of autoimmune diseases.  

Most T cells express an αβ T cell antigen receptor (TCR) on their surface that binds to pathogen-, tumor- or self-derived peptides presented on MHC molecules (pMHC). The TCR is a transmembrane (TM) protein complex composed of non-covalently bound TCRαβ, CD3γε, CD3δε, and CD3ζζ2 dimers. The information of external ligand (pMHC) binding to TCRαβ is conveyed to the signalling network in the cell’s interior through the CD3 dimers. Indeed, the cytosolic tails of CD3 contain cytosolic motifs that enable conversion of the external stimulus to cytosolic signalling cascades leading to activation of T cell. These motifs include: the receptor-kinase (RK) motif that binds to the kinase Lck, immunoreceptor tyrosine-based activation motif (ITAM) that contains tyrosines and a proline-rich sequence (PRS) that can associate with the adaptor protein Nck. Exposure of these motifs that occurs due to pMHC binding results in phosphorylation of the ITAM tyrosines by Lck which then serves as docking sites for signalling proteins with SH2 domains such as ZAP70. ZAP70 then phosphorylates key scaffold proteins such as the transmembrane protein LAT that assemble the so-called signalosome.

The TCR is an allosterically regulated protein complex, in which ligand binding at TCRαβ alters the structure of the CD3 cytosolic tails. In the inactive conformation the TCR’s motifs are not exposed and the cytoplasmic tyrosines are not phosphorylated. In the active conformation, which is stabilized by pMHC or anti-TCR antibody binding, the motifs are exposed, Lck can bind to CD3ε and phosphorylates the ITAM tyrosines of CD3. It was shown that cholesterol binds to the TCRβ TM region, but only to TCRs that are in the inactive conformation, and thereby helps the TCRs to be inactive in resting T cells. Thus, plasma membrane lipid cholesterol is a natural negative allosteric regulator of the TCR that guarantees that in the absence of ligand most TCRs remain in the inactive state.

Besides its function in the allosteric switch of the TCR, cholesterol is involved in TCR signalling by two other mechanisms that are of relevance in the context of this review: (i) it promotes TCR nanoclustering and (ii) it is involved and forming lipid rafts that serve as platforms for signalling.

Cholesterol promotes TCR nanoclustering

TCR can form nanoclusters in which several TCRs are joined to form larger complexes (Fig. 1A). In naïve T cells TCR are mainly expressed as monomeric complexes, whereas effector and memory T cells express the TCR nanoclusters. Importantly, cholesterol together with sphingomyelin promotes this nanoclustering. Indeed, an increase in the cholesterol level of effector and memory αβ T cells results in this enhanced nanoclustering of TCRs and simultaneously increased avidity toward pMHC. Further, removal of cholesterol from T cells results in the disassembly of the TCR nanoclusters. These evidences suggest that cholesterol defines the sensitivity of TCR activation though nanoclustering; a T cell with more and bigger nanoclusters is easier to activate than a cell with predominantly monomeric TCRs. Besides the avidity effect, TCRs within a nanocluster show cooperativity, so that if one TCR is stabilized in the active state by ligand-binding, other TCRs in the nanocluster also reside in the
active state. In this context, cholesterol acts as a positive regulator of TCR by promoting TCR nanoclustering which renders TCR more sensitive towards pMHC stimulation.

Figure 1. The role of cholesterol in autoimmune diseases

A. Effector and memory T cells, such as those being active in autoimmune diseases, contain high levels of cholesterol (yellow squares). This causes TCR nanoclustering and enhanced formation of large lipid rafts. Both lead to strong TCR signalling as a response to stimulation by self-antigens, contributing to autoimmunity.

B. When cholesterol levels are reduced TCR nanoclusters and lipid rafts disassemble, so that TCR signalling is reduced and the autoimmune symptoms are ameliorated.

Cholesterol is involved in the formation of lipid rafts
Depending on their transmembrane region sequence, fatty acid modification and association with other proteins and with the cytoskeleton, plasma membrane proteins localize to specific membrane nanodomains. After stimulation the TCR localizes to nanodomains, called lipid rafts (fig. 1A). This arrangement allows the TCR to get in contact with other important signalling proteins, such as Lck or LAT. In fact, lipid rafts concentrate signalling molecules and thus are important for signalling. In living cells, the lipid rafts are less stable (half-life of microseconds) and much smaller manner (10-40 nm) than anticipated from liposome and detergent experiments. Lipid rafts are enriched in cholesterol and sphingolipids, such as sphingomyelin. Important for the formation of the rafts is an interaction between cholesterol and sphingomyelin. In addition to the dimer, free cholesterol and free sphingomyelin also exist. Disruption of lipid rafts by extraction of cholesterol in vitro and in vivo in mice, dampens TCR signalling, further indicating that these nanodomains are important for signal transduction. Thus, cholesterol is a
positive regulator of TCR signalling by promoting lipid raft formation.

The role of T cell’s cholesterol in SLE
Systemic lupus erythematosus (SLE) is a prototypic systemic autoimmune disease affecting predominantly females in childbearing age. SLE is marked by clinical heterogeneity and can lead to inflammation and tissue damage in multiple organs with skin, joints and kidneys being frequently involved. SLE is characterized by autoantibody production against nuclear self-antigens such as nucleosomes, double stranded DNA and certain ribonucleoprotein particles, resulting from a break of immune tolerance driven by a complex interplay between genetic and environmental factors. Although both innate and adaptive immunity are critically involved in lupus pathogenesis, T cells play a key role and directly drive autoantibody production to nucleosomes. Abnormalities in the T cell compartment of SLE patients comprise profound alterations in TCR signaling that are linked to a hyperactive or exaggerated signaling phenotype. In addition, aberrant T cell differentiation into effector subsets, such as Th17 or Th1 cells is often present, along with an increased production of inflammatory, disease-triggering cytokines.

The molecular mechanisms underlying hyper-responsive TCR signaling are multifactorial, contributing to the heterogeneous disease courses in individual patients. Alterations in the assembly of the TCR signaling machinery and defects in the expression and function of signaling molecules close to the TCR represent one important cause. Among those, a defective expression of the TCR’s CD3ζ chain and alterations in its phosphorylation are frequently found. In these patients, CD3ζ is typically substituted by the Fce receptor type I γ chain (FceRIγ), that recruits Syk instead of ZAP-70, resulting in stronger TCR signaling.

Another important reason underlying aberrant TCR signaling in SLE are lipid dysfunctions that are commonly observed in autoimmune diseases. Generally, lipids are employed by proliferating T lymphocytes to satisfy their energy needs. Hence, lipid dysfunction can skew T cell differentiation and T cell effector function and provoke disease. And as detailed above, an altered lipid composition has an impact on the composition and structure of cellular membranes, for instance on the formation of TCR nanoclusters and lipid rafts. Indeed in SLE, T cells from peripheral blood contain increased levels of cholesterol and glycosphingolipids in the plasma membrane. Consequently in T cells of an healthy individual, lipid rafts are uniformly distributed across the cell membrane whereas, in T cells from SLE patients, these rafts appear to be preclustered and more abundant. TCR nanoclustering was not tested, but is expected to also have been increased. Extraction of cholesterol from the membrane of T cells from SLE patients using methyl-β-cyclodextrin indeed reversed the increased TCR signalling, being in line with reducing TCR nanoclustering and lipid raft amount and size. Likewise, inhibition of cholesterol biosynthesis in SLE T cells by Atorvastatin reduced signaling and T cell activation. The same effect was seen when the inhibitor N-butyldeoxynojirimycin was used, which normalized glycosphingolipid levels in T cells of SLE patients. Also, in a mouse model of lupus, in vivo extraction of cholesterol from T cells by methyl-β-cyclodextrin treatment delayed disease onset. Vice versa, clustering of lipid rafts in T cells by cholera toxin B could promote disease progression in vivo. Thus, lowering the content of membrane cholesterol or normalizing glycosphingolipids, disrupts TCR nanoclusters and lipid rafts, going along
with a restoration of normal TCR signalling and ameliorated T cell function with beneficial effects on lupus pathology.

**Conclusions**
Hyperlipidaemia and dysregulated lipid metabolism not only increase cardiovascular risk, but are also involved in the pathogenesis of autoimmune diseases. Among broad effects on various immune cells, lipid dysregulation importantly impacts T cell function and TCR signalling. Given their central role in lupus pathogenesis, targeting lipids in T cells might be an effective strategy to reinforce self-tolerance and weaken autoimmunity.

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