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Autoimmune disease development based on possible mislocalization of intracellular and extracellular proteins

Borros Arneth*

Justus Liebig University Giessen, Giessen, Germany

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ABSTRACT

Background: T helper cells can differentiate into several subsets of T lymphocytes, including Th1, Th2, and regulatory T (T_{reg}) cells. As a result of this ability to differentiate, the corresponding T cell receptor (TCR) spectra display considerable cellular plasticity and interchangeability. In contrast, T lymphocyte differentiation and separation into $CD4^+$ and/or $CD8^+$ T cell lines creates stable populations over a person's lifetime, which abrogates the plasticity and interchange between these cell types and their corresponding TCR spectra but results in considerable stability regarding the corresponding TCR sequences and spectra. This separation of TCR spectra agrees with the well-known concept of major histocompatibility complex class (MHC) restriction. Therefore, $CD4^+$ and $CD8^+$ T cell populations possess different (but stable) TCR spectra, which present differences in antigens between intra- and extracellular space. Thus, mislocalization can lead to autoimmunization and the development of autoimmune disease.
Methods: To test this hypothesis, human intra- and extracellular proteins and intra- and extracellular extracts were incubated overnight with whole-blood samples from the same subject, and the following day, a cell proliferation assay based on bromodeoxyuridine (BrdU) incorporation was performed.
Results: The BrdU assay showed that the addition of intracellular proteins and extracts to the mixture resulted in significantly greater cell proliferation after overnight incubation, whereas significantly less proliferation was obtained with addition of extracellular proteins and extracts (plasma).
Conclusions: These results support the proposed hypothesis and show that hidden antigens are present in and released with intracellular proteins. Furthermore, both albumin and insulin activated $CD4^+$ and $CD8^+$ lymphocytes in a concentration-dependent manner. At low concentrations (<0.1 μg/ml), both proteins showed the ability to inhibit $CD4^+$ and $CD8^+$, whereas at high concentrations (>1000 μg/ml), both proteins activated $CD4^+$ and $CD8^+$ T lymphocytes.

1. Introduction

Zinkernagel and Doherty first reported the viewpoint that cytosolic proteins bind to major histocompatibility complex class I (MHC I) receptors and are subsequently presented to $CD8^+$ T lymphocytes, whereas T helper cells ($CD4^+$ T cells) recognize peptides bound to MHC II receptors through T cell receptors (TCRs) and CD4 protein (Zinkernagel and Doherty, 1974a,b). These two pathways are used to recognize the peptides produced by the body and to identify foreign peptides on antigen-presenting cells (Zinkernagel and Doherty, 1974a).

Although these two basic theorems, collectively known as MHC restriction, are widely accepted, the medical consequences of this biochemical and cellular differentiation of the entire "antigen repertoire" or "antigen spectrum" into two parts have not been fully considered. As reported by Zinkernagel and Doherty (1974a,b), antigen-presenting cells bind and internalize foreign extracellular proteins and display peptides formed from the digestion of these proteins on class II MHC proteins, whereas peptides derived from cytosolic proteins are bound to class I MHC proteins.

Cytotoxic T cells recognize foreign peptides presented by class I MHC proteins with the aid of the coreceptor CD8 (Fig. 1A). Peptides derived from cytosolic proteins are bound to MHC I molecules and then presented and recognized by $CD8^+$ T lymphocytes (Fig. 1B). $CD4^+$ T cell receptors interact with class II MHC molecules in a manner analogous to the way $CD8^+$ T cell receptors interact with class I MHC molecules (Fig. 1A). However, helper T cells and cytotoxic T

* Correspondence to: Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, University Hospital of the Universities Giessen and Marburg UKGM, Campus Giessen, Justus Liebig University Giessen, Feulgenstr. 12, 35392 Giessen, Germany. Campus Marburg, Baldingerstr. 1, 35043 Marburg.
E-mail address: borros.arneth@klinchemie.med.uni-giessen.de

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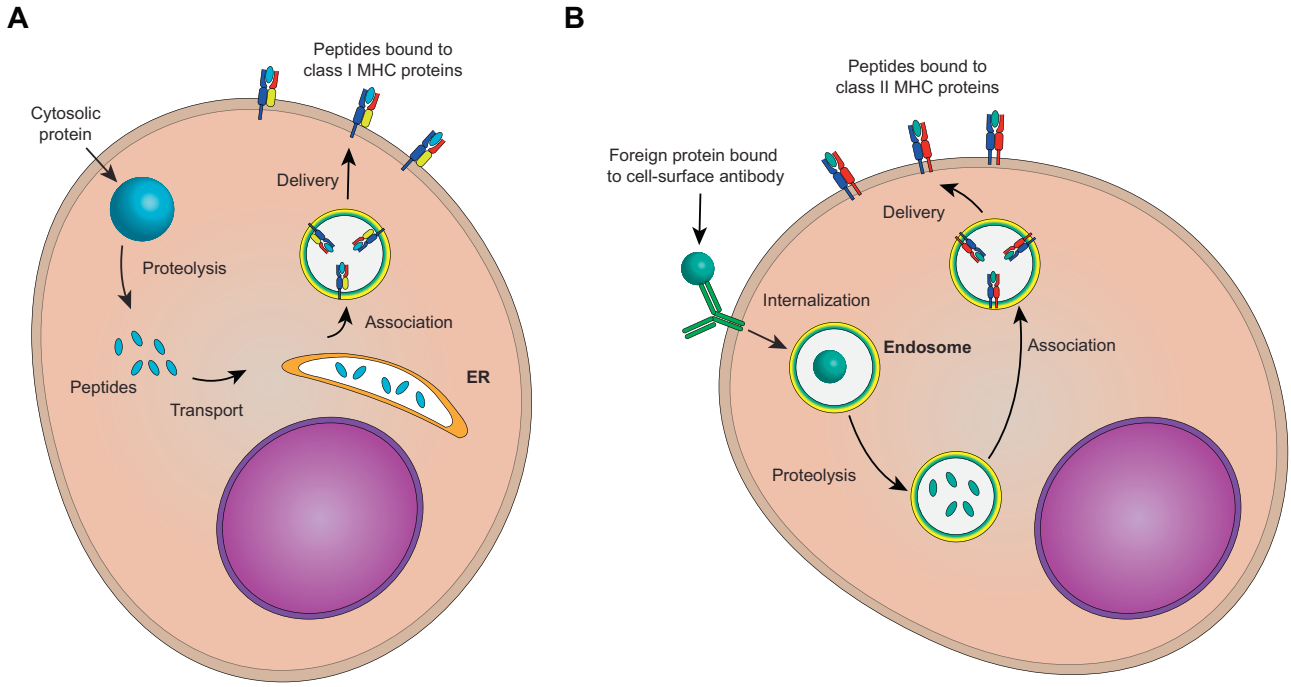

Fig. 1. Two mechanisms of antigen processing lead to a division of the antigenic spectra of CD4 and CD8T lymphocytes. (A) Peptides derived from cytosolic proteins. Class I MHC proteins on the surfaces of nearly all cells display peptides derived from proteasome-assisted proteolysis of cytosolic proteins. (B) Peptides derived from extracellular proteins. An antigen-presenting cell bound and internalized foreign proteins and displayed peptides obtained from the digestion of these proteins on class II MHC proteins. Figs. 1A and B are adapted according to Zinkernagel and Doherty (1974a,b).

cells are distinguished by other proteins that are expressed on their surfaces.

This separation of $CD4^+$ and $CD8^+$ TCRs corresponds to the MHC restriction identified by Zinkernagel and Doherty at the T cell level. In particular, helper T cells express CD4 protein instead of CD8. CD4 consists of four immunoglobulin domains extending from the T cell surface and a small cytoplasmic region. This study focused on the consequences of MHC restriction on T cells and subsequently on the antigenicity of the body's own peptides and proteins.

1.1. Theory

It is thought that helper T cells express CD4 protein to recognize foreign (extracellular) peptides on antigen-presenting cells. Consequently, the antigen repertoire, or antigen spectrum, is separated into two parts, each associated with a different T cell population. The antigen repertoire includes TCRs, which specialize in recognition of cytosolic peptide structures. Therefore, cytosolic self-peptides are presented on MHC I and are recognized by TCRs with the help of the CD8 protein by $CD8^+$ T cells. In addition, the TCR repertoire has an extracellular component. Specifically, extracellular peptides are presented on MHC II receptors and are recognized by TCRs on $CD4^+$ T cells using CD4 protein. The research described herein led to the hypothesis that the cytosolic peptide structures produced by the body (or, more precisely, their complementary binding structures) should be "missing" from the TCR spectra of $CD8^+$ T cells and therefore not recognizable, whereas physiological extracellular peptide structures should be present and recognizable. Furthermore, the present study proposed that physiological extracellular proteins should be missing from the TCR spectra of $CD4^+$ T cells, whereas physiological cytosolic peptide structures should be present and recognizable.

The logical consequence of these hypotheses is that physiological intracellular proteins are recognized by TCRs on $CD4^+$ T cells, whereas their recognition-specific patterns are absent from TCRs on $CD8^+$ T cells. Conversely, physiological extracellular proteins are recognized by TCRs on $CD8^+$ T cells, whereas their recognition-specific patterns are absent from TCRs on $CD4^+$ T cells.

This theory provides a completely new viewpoint that has significant effects on understanding how autoimmune disease develops. The best approach to test this theory would be through clinical studies of such disorders. The theory predicts that an innate intracellular protein will be recognized as a foreign body if it arises or accumulates extracellularly. Through similar reasoning, an extracellular protein will be recognized as a foreign body if it arises or accumulates intracellularly. In other words, the localization of a specific protein plays a substantial role in antigenic determination in the context of whether an autoimmune reaction develops toward the misplaced protein.

Changing the localization of a specific protein is therefore critical. This theory predicts that an autoimmune disease will develop if changes in protein localization occur continuously within the body, either rapidly or slowly. This theory has been presented to the scientific community at several congresses, including the Frontiers in Immunology Research Network (FIRN) congresses in Hawaii and Athens and the German Society of Immunology (DGfI) congress in Leipzig, and has also been reported in several publications (Arneth, 2003, 2004; Arneth and Birklein, 2009; Arneth, 2009). As a result, the validity of the role of protein mislocalization in the development of autoimmunity is becoming more widely considered.

2. Materials and methods

To test the above-described hypotheses in detail with various human proteins, human albumin and human insulin were added to human whole-blood samples from healthy subjects (N = 12) as extracellular proteins, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and ß-actin were added as intracellular proteins. All participants provided informed consent, and the study was approved by the review board of the University of Mainz, Germany.