UNIVERSITY OF AMSTERDAM

UvA-DARE (Digital Academic Repository)

Tuning the show

Genetic engineering to unravel antigen presentation by the human leukocyte antigen class I

de Waard, A.A.

Publication date
2021

Link to publication

Citation for published version (APA):
de Waard, A. A. (2021). *Tuning the show: Genetic engineering to unravel antigen presentation by the human leukocyte antigen class I*. [Thesis, externally prepared, Universiteit van Amsterdam].

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

UvA-DARE is a service provided by the library of the University of Amsterdam (https://dare.uva.nl)

Download date:20 Jun 2024

ENGLISH SUMMARY

Presentation of peptides by the Human Leukocyte Antigen class I (HLA-I) is critical for the CD8$^+$ T cell response during cancer and infection. Each CD8$^+$ T cell is equipped with a unique receptor, called the T cell receptor. This receptor recognizes a specific peptide in complex with HLA-I presented on every cell in the body. Due to negative selection of CD8$^+$ T cells specific for self-peptides, most CD8$^+$ T cells only recognize non-self-peptides in the context of HLA-I. Cells with DNA mutations or foreign DNA, such as tumor cells or infected cells, can produce non-self-peptides and present these to CD8$^+$ T cells in HLA-I. After recognition, a CD8$^+$ T cell can unleash its cytotoxic activity to kill the target cell.

During evolution, HLA-I genes duplicated and acquired many mutations. As a result, each individual carries 3 maternal and 3 paternal classical HLA-I genes: HLA-A, HLA-B and HLA-C. Each of these is highly polymorphic throughout the population. Due to the polymorphic sites, which are mainly located in the peptide-binding groove, each HLA-I allele presents its own unique peptide repertoire to CD8$^+$ T cells. The advantage of this system is that peptides derived from a pathogen that fail to be presented by HLA-I alleles in one individual may be presented by an HLA-I allele in another individual. As a result, the susceptibility of a complete population to a single pathogen is prevented.

HLA-I molecules are synthesized, folded and loaded with peptide in the endoplasmic reticulum (ER). During synthesis, a glycan is attached to the asparagine at position 86 by the oligosaccharide transferase-complex and is immediately trimmed by the subsequent actions of glucosidase I and α-glucosidase II. This allows successive binding of the lectin chaperones calnexin (CNX) and calreticulin (CALR). After association of beta-2-microglobulin (B2M) with HLA-I, it is recruited to the peptide loading complex (PLC). This complex additionally consists of tapasin, ERp57 and the transporter associated with antigen processing (TAP, consisting of TAP1 and TAP2). Peptides generated in the cytosol by the proteasome are shuttled into the ER by TAP and possibly trimmed by ER aminopeptidases (ERAP1 and ERAP2). Peptides that meet the requirements for binding an available HLA-I molecule are then loaded onto HLA-I. The peptide/HLA-I/B2M trimer is then released from the PLC and shuttled to the cell membrane where it presents the peptide to CD8$^+$ T cells.

Tumor cells with a dysregulated antigen presentation machinery benefit from less CD8$^+$ T cell-mediated immunological pressure and may also be insensitive to immunotherapeutic strategies that aim to (re)activate tumor-specific CD8$^+$ T cells. Knowledge of functional HLA-I antigen presentation is indispensable to understand and address tumor immune escape. Identification of the consequences of such dysregulation for functional HLA-I antigen presentation can contribute to the design of novel therapies that aim to restore HLA-I function or specifically target (peptide/)

HLA-I antigens that are not affected by the dysregulation. With the arrival of novel genetic engineering strategies, such as CRISPR/Cas9, important questions in the field of HLA-I antigen presentation can be addressed. In this thesis, we exploited various genetic modification strategies to improve the knowledge on known factors that contribute to HLA-I antigen presentation and extend current knowledge by identification of novel factors contributing to functional HLA-I antigen presentation.

In **Chapter 2** we present a novel panel of HLA-I antigen presentation machinery knockout cells, termed PAKC, to facilitate novel studies into HLA-I antigen presentation. This panel consists of 10 cell lines from the same genetic origin. Each cell line is made genetically knockout for another gene involved in the HLA-I antigen presentation pathway. We validated the knockout of the respective genes on a genetic and protein level and characterized the cell lines for their HLA-I surface expression. In addition, we demonstrate how these cell lines can contribute to novel studies on the functional interplay between antigen presentation machinery and their role in HLA-I antigen presentation.

A novel study demonstrating the application of these cell lines is presented in **Chapter 3**. Peptides that are presented independently of TAP are currently being investigated as novel immunotherapeutic targets for TAP-deficient tumors. Using the wild type and TAP1 knockout cell lines from the panel described in Chapter 2, we identify a peptide that is presented independently of TAP on both TAP-deficient and TAP-proficient cells. Our data indicates that various, but not all healthy cell types present this antigen in sufficient concentrations to induce an in vitro T cell response of T cells specific for this TAP-independent peptide. Depending on the immunotherapeutic strategy, targeting peptides that are presented irrespective of the TAP-status of a cell may lead to T cell reactivity towards healthy cells or will be inefficient due to the negative selection of self-reactive T cells. These data demonstrate the necessity of extensive testing of a broad panel of healthy cell types to define clinically relevant TAP-independent peptides suitable for immunotherapeutic targeting.

Another study utilizing various cell lines from PAKC is described in **Chapter 4**. Since HLA-I is highly polymorphic, the dependency of individual HLA-I alleles on the peptide loading machinery may differ. This has been described for tapasin and TAP, but by studying the surface expression of 10 different HLA-I alleles in the cell lines deficient for one of the components of the PLC from Chapter 2, we demonstrate that HLA-I alleles have a distinct dependency on each of the PLC components. These data warrant further investigation into properties of HLA-I alleles and the individual contributions of the PLC components to HLA-I antigen presentation.

As a prelude to Chapter 6, where the role of glycosphingolipids (GSLs) will be discussed in the context of antigen presentation, we review the knowledge of GSLs in the immune system in **Chapter 5**. We first summarize the current knowledge on the GSL repertoire expressed by the major immune cell subtypes in different stages

of differentiation. This summary is followed by a review of functions that have been attributed to GSLs in- and outside the immune system. Lastly, we hypothesize on possible other functions of GSLs on immune cells.

In **Chapter 6**, we then identify a novel role for a specific type of GSLs, called neolacto-series GSLs (nsGSLs), in the immune system. Using genome-wide insertional mutagenesis screening, we discovered that HLA-I on the surface of cells lacking the ER- and Golgi-resident intramembrane protease SPPL3 was less accessible for antibodies than on cells that expressed SPPL3. We find that the activity of the key enzyme in nsGSL synthesis, namely the glycosyltransferase B3GNT5, is enhanced in the absence of SPPL3. Sialylated nsGSLs are capable of interfering with antibody binding to HLA-I, but more importantly, also downmodulate a T cell response. Thus, upregulation of sialylated nsGSL synthesis by tumor cells is possibly a mechanism to evade T cell mediated immunity. In glioma, downregulation of SPPL3 and upregulation of B3GNT5 expression correlates with a decreased overall patient survival, suggesting that the increase in nsGSLs is indeed beneficial for the tumor. Pretreatment of nsGSL-rich tumor cells with FDA-approved GSL synthesis inhibitors resulted in an increased T cell response against the tumor cells, demonstrating that GSL synthesis inhibitors may thus provide a viable therapy for patients with tumors that display excess nsGSLs.

The most important results and considerations of this thesis are further discussed in **Chapter 7**. There is still a large unexplored territory in HLA-I antigen presentation for which we think PAKC is a suitable model to accelerate research, for example for studying the surface expression and peptide repertoire of different HLA-I alleles in the various cell lines. We discuss the possible layers of regulation of SPPL3 activity and the mechanism by which nsGSLs alter the accessibility of membrane receptors. Furthermore, we hypothesize on a biological function of nsGSLs as modulators of membrane receptor function. The research presented in this thesis thus increases knowledge of the known factors in HLA-I antigen presentation, and reveals novel layers of regulation contributing to functional HLA-I antigen presentation.