VAR2CSA Epitope Identification – Finding All the Needles in All the Haystacks

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Published in:
Scandinavian Journal of Immunology

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

HLA-A*24:02 and HLA-B60, respectively. Tetramer staining confirmed that the optimal epitopes were YYNAFHWAI and RESEEESVSL presented on HLA-A*24:02 and HLA-B*40:01 (a member of the previously designated HLA-B60 specificity), respectively. PBMCs obtained post HCT from five other male recipients of female donor grafts have been analysed for T-cell responses with ELISpot. Responses have been observed and further analysis is ongoing.

Conclusion : Using a HLA-tetramer approach to identify the optimal epitopes of two known mHags encoded by the Y-chromosome as well as the presenting HLA restriction elements at high resolution, we have demonstrated the feasibility of a reverse immunology approach in mHag discovery.

Disclosure of Interest: None Declared.

SSI13-1129

Endogenous and Natural Complement Inhibitor Attenuates Myocardial Injury and Arterial Thrombogenesis

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Background: Coagulation disorders and reperfusion of ischemic myocardium are major causes of morbidity and mortality. Lectin pathway initiation complexes are composed of multimolecular carbohydrate recognition subcomponents and three lectin pathway specific serine proteases. We have recently shown that the lectin pathway specific carbohydrate recognition subcomponent mannose-binding lectin (MBL) plays an essential role in the pathophysiology of thrombosis and ischemia/reperfusion injury. Thus, we hypothesized that the endogenous MBL associated protein, MAP-1, that inhibits complement activation *in vitro* also could be an *in vivo* regulator by attenuating myocardial schema/reperfusion injury and thrombogenesis when used at pharmacologic doses in wild type mice.

Methods and Results: MAP-1, in two mouse models, preserves cardiac function, decreases infarct size, decreases C3 deposition, inhibits MBL deposition and prevents thrombogenesis. Further, we also demonstrate that MAP-1 displaces MASP-1, MASP-2 and MASP-3 from the MBL complex.

Conclusions: Our results suggest that the natural, endogenous inhibitor, MAP-1effectively inhibits lectin pathway activation *in vivo*. MAP-1 at pharmacologic doses represents a novel therapeutic approach for human diseases involving the lectin pathway and its associated MASPs.

Disclosure of Interest: None Declared.

SSI13-1153

VAR2CSA Epitope Identification – Finding All the Needles in All the Haystacks

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Pregnancy Associated Malaria (PAM) is a major cause of mother and child birth-associated fatalities in Sub-Saharan Africa. PAM is caused by infection with the mosquito borne protozoan Plasmodium falciparum parasite. Evading immune detection by hiding inside erythrocytes (ECs), the parasite is able to sequester in the placenta. This is mediated through interaction between the EC membrane bound tissue-specific antigen VAR2CSA on the surface of the EC and chondroitin sulphate A (CSA) receptor on the endothelium of the microvasculature. Immunity to PAM is IgG mediated and gradually acquired through parity dependent exposure to clonal VAR2CSA variation. VAR2CSA is considered the primary PAM vaccine candidate.

Epitope identification is a crucial part of vaccine development. However assaying peptides manually is a cumbersome, costly and time consuming process. Here we have applied a novel high-density peptide microarray for epitope identification in the Plasmodium falciparum VAR2CSA variants FCR3 and 3D7.

All FCR3/3D7 15-mers were assayed with antibodies purified from sera from 24 distinct immunizations yielding 24 sectors. Positive and negative controls were FCR3 c-terminus located V5 and the 15-mer flanking peptide GAGA… respectively. Two methods were applied for data analysis: Direct signal mapping and non-parametric statistical n-mer evaluation.

Positive and negative controls were identified correctly. The strongest pan-sector signal was identified in the proximity of CGSARTMKRGYKNDNYELC. This peptide was experimentally found to raise a significant response, however not inhibitory.

We have shown how linear epitope scan of large antigens can be performed relatively cheap and fast. Our analysis was able to correctly assign positive and negative controls

along with suggestion a novel reactive peptide. VAR2CSA exhibits surprisingly few linear epitopes and further work is required in order to fully understand and describe the antigenicity of VAR2CSA.

Disclosure of Interest: None Declared.

SSI13-1113

Attempt to Vaccinate Horses Against Insect Bite Hypersensitivity: Comparison of Injection Sites using Recombinant Allergens with or without IC31® Adjuvant

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Background: Insect bite hypersensitivity is an allergy of type I in horses with production of IgE. It is caused by bites of *Culicoides* spp. also called biting midges that do not exist in Iceland. All horse breeds can be affected but horses born in Iceland and exported to the continent are more strongly affected than Icelandic horses born abroad. We have isolated the respective *Culicoides* allergen genes and expressed the proteins in *E. coli*.

Objectives: The objective was to compare intradermal and intralymphatic vaccinations, using four purified recombinant allergens, with and without Th1 focusing adjuvant. The final aim is to develop immunotherapy against insect bite hypersensitivity.

Methods: The allergens, originated from the salivary glands of *C. nubeculosus*, were produced in *E. coli* and purified. The adjuvant, IC31® was from Intercell, Vienna. Twelve healthy Icelandic horses were vaccinated three times with four recombinant allergens (Culn1, Culn2, Culn5 and Culn9). Six horses were injected intralymphatically, three with IC31® and three without and six were injected intradermally, three with IC31® and three without. PBMCs were stimulated *in vitro* for measurement of cytokines in qRT-PCR and serum was collected for analysis of antibody response in western blot and ELISA. The horses were tested for allergy in sulfidoleukotriene release test and in intradermal skin test.

Results: The allergens in IC31® adjuvant gave much stronger specific IgG response to the allergens. Intralymphatic injection was slightly more efficient than intradermal. The IgG induced against all four allergens was mainly IgG1 and IgG4/7 and to a lesser extent IgG5. The IgG antibody generated after the vaccination was able to partly block binding of serum IgE from horses with insect bite hypersensitivity. The horses did not make specific IgE. There was no indication of induction of IgE-mediated reactions, as they did not respond to *Culicoides* extract stimulation in a sulfidoleukotriene release test and not to the recombinant allergens in skin test.

Conclusions: Vaccination of horses both intralymphatically and intradermally with purified allergens in IC31® adjuvant induced immune response without adverse effects and without IgE production. The horses were not sensitized and produced IgG that could inhibit allergen-specific IgE binding. We therefore conclude that both the injection methods and the IC31® adjuvant are strong candidates for further development of immunotherapy in horses.

Disclosure of Interest: None Declared.

SSI13-1027

Vaccine Induced Immunotherapy of CMV Infection

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Cytomegalovirus (CMV) is a β-herpesvirus, which is carried asymptomatically by a large fraction of the human population. However, in case of immune suppression, such as AIDS, organ transplantations and in particular bone marrow transplantations, CMV infections can reactivate and cause substantial damage to lungs, liver and intestine. It is therefore imperative to develop protective measures for bone marrow transplantation recipients, very likely in the form of vaccination.

In the planned project, we will investigate if vaccination with recombinant adenovirus can induce a protective effect against infection with CMV, and if such a vaccine can be used in a preemptive or therapeutic manner in association with bone marrow transplantations. To assert the potential protective capabilities of a CMV vaccine, we will focus on the murine CMV (mCMV) model with primary and latent mCMV infections in relation to bone marrow transplantation. Additionally, we will investigate the protective potential of adaptive transfer of vaccine induced, mCMV-specific $CD8^+$ memory T cells to immune suppressed recipients. If successful, these studies would provide the intellectual framework for future development of a vaccine to be used in association with CMV recurrence after bone marrow transplantation and perhaps other immune suppressive scenarios.

Disclosure of Interest: None Declared.