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Review

A recombinant human HLA-class I antigen linked to dextran elicits innate and adaptive immune responses

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ABSTRACT

The objective of this study was to produce and evaluate the immunogenic potential of a recombinant HLA-class I antigen linked to dextran. The HLA-A*0201 heavy chain and β2 microglobulin were cloned by PCR amplification of overlapping oligonucleotides and produced in *E. coli*. These were assembled with a CMV binding peptide motif, the HLA complex was biotinylated and bound by streptavidin coated dextran at a ratio of 24 HLA to 1 dextran molecule (termed Dextramer). Allostimulation of human PBMC *in vitro* and *in vivo* immunization of Balb c mice with the HLA-A*0201 construct elicited $CD4^+$ and $CD8^+$ T cell proliferative responses, IgG specific antibodies in mice and in human T cell proliferation and APOBEC3G mRNA. These adaptive and innate immune responses induced by a novel recombinant HLA construct in human cells and mice suggest their application as a potential vaccine candidate against HIV infection.

Contents

Abbreviations: A3G, APOBEC3G;CMV, cytomegalovirus;DC, dendritic cells;FITC, fluorescein isothiocyanate;HIV, human immunodeficiency virus;HLA, human leukocyte antigens;SIV, simian immunodeficiency virus;HSP, heat shock protein;TH1, T helper 1.

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1. Introduction

Immunization of macaques with SIV grown in human $CD4^+$ T cells (Desrosiers et al., 1989; Murphey-Corb et al., 1989; Stott et al., 1990; Carlson et al., 1990; Hunsmann et al., 1995; Biberfeld and Putkonen, 1995) or with the cells alone (Stott, 1991; Stott et al., 1994; Arthur et al., 1995; Chan et al., 1995) yielded about 85% and 55% protection against SIV infection, respectively. The protection was dependent on HLA antigens acquired by the virions in the process of budding through the human $CD4^+$ T cell membrane in which the SIV was grown. Despite the reproducibility of preventing SIV infection in macaques, this approach was abandoned largely because of the potential adverse effects of immunization with HLA^+ cells, and using $CD4^+$ T cell lines propagated with oncogenic viruses.

There is also evidence in humans that alloimmune responses can prevent HIV-1 infection. This was demonstrated *in vitro* by inducing cytotoxic lymphocytes and soluble factors (Shearer et al., 1993). Systemic *in vivo* alloimmunization of women revealed that HIV-1 replication in $CD4^+$ T cells *ex vivo* is inhibited (Wang et al., 1999). Epidemiological evidence suggests that transmission of HIV from mother to baby occurs more frequently among uniparous women and mother–child HLA-class I concordance increases perinatal HIV-1 transmission (McDonald et al., 1998). Furthermore, sera from multiparous women may contain alloantibodies and CCR5 antibodies which inhibit *ex vivo* HIV-1 replication (Wang et al., 2002a). Indeed, alloimmunization has been proposed as a strategy for inducing immune protection against HIV infection (Lehner et al., 2000).

The objective of this study was to evaluate the immunogenic potential of recombinant HLA-class I antigen (A*0201) linked by the biotin–streptavidin method to dextran (Dextramer) and to use HSP70 as an adjuvant to elicit immune responses. Immunization of Balb c mice with the HLA-A*0201 Dextramer construct elicited an increase in both $CD4^+$ and $CD8^+$ T cell proliferation and IgG antibodies to the HLA-A*0201 dextramer. Human $CD4^+$ and $CD8^+$ T cell proliferative responses and an innate immune response were induced *in vitro* by the Dextramer stimulating upregulation of APOBEC3G mRNA in human $CD4^+$ T cells.

2. Materials and methods

2.1. Cloning and production of HLA-A*0201 heavy chain

The human MHC-class 1 HLA-A*0201 coding sequence minus the signal peptide and transmembrane regions was obtained from GenBank (acc # M84379) by backtranslation. Codon usage was optimized to *E. coli* using www.entelechon.com before the gene was synthesized by PCR using 10 overlapping DNA primers (DNA Technology, Denmark) and KOD polymerase (EMD Chemicals, Novagen). The sequence was verified by repeated DNA sequencing (MWG Biotech, Ebersberg, Germany) and base errors introduced by the PCR were corrected using Quick Change multi site-directed mutagenesis kit (Stratagene, La Jolla, CA) before cloning in pGarboczi (Garboczi et al., 1992).

Recombinant HLA-A*0201 was produced by *E. coli* batch fermentation. Bacteria were harvested by centrifugation and resuspended in ice cold buffer (50 mM Tris–HCl pH 8.3, and 150 mM NaCl). Proteinase inhibitor AEBSF was added to a final concentration of 0.5 mM before lyses of the cells by cell disruption. Inclusion bodies were isolated from the cell lysate by centrifugation (20 g rpm/4 °C) and washed thoroughly three times in ice cold buffer (2 M urea, 2% Triton X-100, 0.5 M NaCl, and 20 mM Tris–HCl pH 8.0). After the final wash the inclusion body pellet was resuspended 8 M Urea, 150 mM NaCl, and 20 mM Tris–HCl pH 8.0). Undissolved material was removed by centrifugation and the supernatant was filtered through 0.2 μm filter before loading on to an ion exchange column (Q Sepharose fast flow). The HLA heavy chain was eluted by a 0–100% gradient of 8 M Urea, 500 mM NaCl, and 20 mM Tris–HCl pH 8.0. Relevant fractions were identified by SDS-PAGE and concentrated.

2.2. Cloning and production of human β2 microglobulin

Cloned human β2 microglobulin (GenBank acc # CAG33347.1) was a kind gift from L. Østergaard (Danish Cancer Society). Recombinant β2M was produced by *E. coli* batch fermentation. Bacteria were harvested by centrifugation and resuspended in ice cold buffer (50 mM Tris–HCl pH 8.3, and 150 mM NaCl). Proteinase inhibitor AEBSF (Sigma, St. Louis) was added to a final concentration of 0.5 mM before lyses of the cells by cell disruption. Inclusion bodies were isolated as described above for the heavy chain. Undissolved material was removed by centrifugation and the supernatant was filtered through 0.2 μm filter before loading on to an IMAC column. The β2M preparation was then washed in 125 mM NaCl, and 20 mM Tris–HCl pH 8.0 before elution by an imidazole gradient (0–100%, 500 mM imidazole, 125 mM NaCl, and 20 mM Tris–HCl pH 8.0). The relevant β2M fractions were identified by SDS-PAGE, sterile filtered and concentrated before gel filtration (column Hi-Load 26/60 Superdex 75) was performed to isolate the monomeric β2M from dimeric and multimeric forms.