SUMMARY

Three synthetic peptides corresponding to epitopes from outer membrane and secreted proteins from *Neisseria meningitidis* were synthesized by solid-state Fmoc chemistry. The peptides were a 20-mer from the Opc invasin [175], as well as a 20- and 50-mer of IgA1-protease [150].

The peptides were coupled to bovine serum albumin, ovalbumin, keyhole limpet hemocyanin, thyroglobulin and tetanus toxoid via a C- or N-terminal cysteine. Aminooxyacetyl derivatives were condensed on a synthetic ring template to yield tetra-oximes. The peptides were also incorporated into liposomes and the 20-mers were synthesized in the form of tetra-lysine multiple antigenic peptides (MAPs).

Immunogen synthesis was paralleled by the molecular characterization of the various constructs employing mass spectrometry, amino acid analysis and chromatographic methods.

Balb/c mice were immunized 3 times with normalized formulations of all of these constructs, as well as with free peptides or with peptides in the presence of cytokines IL-4 and GM-CSF. Immunizations were performed in the presence of Freund's Complete and Incomplete Adjuvants, except for liposomes.

Immunogenicity was evaluated by sub-class-specific ELISPOT analysis and by ELISA. The results show dramatic differences in immunogenicity, depending on the immunogen type:

The tested protein carriers confirmed their standing as a general, straightforward and reliable approach for increasing immunogenicity of synthetic peptide antigens. As little as 200 pmol antigenic peptide and 2.2 μg protein carrier per injection efficiently induced an immune response with the 3-fold immunization regime.

The protein carriers were not equally immunogenic. KLH, thyroglobulin and tetanus toxoid demonstrated superior immunogenicity over BSA and ovalbumin This observation raises doubt about the general use of these latter proteins as immunogenic carriers for peptide immunization practice. High molecular weight of a protein carrier resulting in high peptide to carrier coupling ratios appear to be beneficial for immunogenicity.

As demonstrated for an ovalbumin carrier conjugate, a molar coupling ratio of 1.4 sufficed for efficiently stimulating a murine immune response, i.e. a minimum representation of one antigenic peptide ligand per carrier molecule proved to be immunogenic.

The results also reflected an antigen/carrier interdependence, i.e. conjugate immunogenicity depends both on the carrier and on the peptide antigen. None of the protein carriers excelled as a standard approach.

Non-protein formulations (free peptides, liposomes, MAPs and tetra-oximes) required the peptide ligand to be itself immunogenic in order to obtain immunostimulatory potency: Among the three synthetic peptides investigated, only the

50 aa peptide of IgA1-protease (IgA1-PA50) induced elevated IgG serum titers if administered as free peptide and only that peptide showed increased immunogenicity upon integration into liposomes and tetra-oximes thereafter. The tetra-oxime type 25 kDa “synthetic protein” of peptide IgA1-PA50 was comparable to BSA and ovalbumin protein conjugates concerning immunostimulatory potency. Structural analysis of IgA1-PA50 revealed the presence of high proportions of stable, ordered structure for this particular peptide. Moreover, the observed IgG class-switch in IgA1-PA50 peptide immunization is indicative for the presence of at least one T cell epitope on the 50-mer. By T cell epitope mapping, human but not known murine T cell epitopes could be identified.

Two recombinant murine cytokines which were tested as adjuvants, Interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF), had only minor effects on peptide antigen immunogenicity.