

HyperCarrier™: A Highly Immunogenic Carrier Protein

Antibodies, in particular the production of specific antibodies, have been essential in the advancement of many scientific fields, particularly proteomics, immunology and cell biology.

A common practice for the generation of specific antibodies is the use of immunogenic peptides derived from the protein of interest. These peptides are short sequences of amino acids that are antigenic, however they lack elements required for T cell activation. Activation is achieved with the use of "carrier proteins" that are coupled to the small antigenic peptide.

The Immune Response

The coupling of the peptide to a carrier protein is important in the stimulation of the immune response and the generation of antibodies. Below is a brief description and diagram (Figure 1) summarizing the immune pathway responsible for detecting the peptide and carrier protein and generating subsequent antibodies.

The major histocompatibility complex (MHC) II pathway is responsible for the detection and generation of antibodies against foreign material, such as the carrier protein:peptide complex.

A B-cell or a circulating macrophage (antigen presenting cell (APC)) travels through the host's blood stream in search of foreign material (carrier protein:peptide complex)(1). Once detected, the macrophage captures the carrier protein:peptide complex and brings it inside the cell, by a process known as endocytosis (2). The endosome, containing the carrier protein:peptide complex, moves towards the center of the cell and becomes acidified (3), at which time it may fuse with the lysosomes, which contain acid proteases. The acid proteases, in the acidified endosomes and lysosomes, digest the complex into small peptides (4).

The immature MHC class II (MHCII) proteins wait for the detection of foreign material in the endoplasmic reticulum, where it is in a complex with the Invariant chain (Ii) (5). Upon detection of the foreign material, the MHCII:Ii complex leaves the ER, traverses the Golgi and endosomes and finally fuses with the MHCII compartment (6). Cathepsins, in the MHCII compartment, partially digest Ii chain leaving CLIP in the peptide-binding site.

The endosome/lysosome, with the carrier protein:peptide peptides, fuses with the MHCII compartment and a human leukocyte antigen (HLA) protein replaces CLIP with a carrier protein:peptide peptide (7). The mature, peptide-loaded MHC class II protein moves to the plasma membrane and is presented on the cell surface (8).

A specific helper T-cell binds the peptide-loaded MHC class II protein through its T-cell receptor and CD4 receptor (9), which stimulates the macrophage to release interleukin-1 (IL-1). In turn, the helper T-cell releases IL-2 that stimulates itself and the macrophage (10). This stimulation and release of further interleukins and lymphokines leads to the proliferation of the macrophages and the helper T-cells, producing clones and some memory helper T-cells (important for fast secondary response). The released interleukins stimulate B lymphocyte cells (11) to initiate mitosis and cloning (12), producing more B lymphocyte cells and some memory B-cells (important for fast secondary

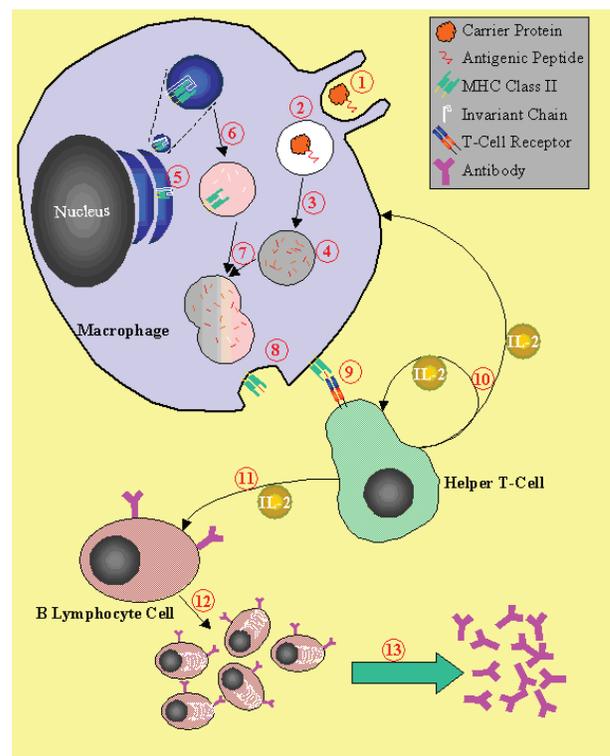


Figure 1: The Immune Response initiated by a Carrier protein:peptide complex.
For legend see text.



think proteins! think G-Biosciences!



response). After cloning, the B lymphocyte cells enlarge and develop an extensive endoplasmic reticulum and then begin mass-producing antibodies (13).

Carrier Proteins

Many proteins are suitable for the role as a carrier protein and it is their properties that determine, to a large extent, the immune response and outcome of antibody production. Several factors are important to consider in the choice of the carrier protein. The first is the size of the carrier protein. Larger proteins (>60kDa) are preferable as it is highly probable that they contain the elements required for T-cell activation and they have multiple and sufficient numbers of exposed residues for peptide coupling, such as amine and sulfhydryl groups. An additional important factor in the choice of a carrier protein is to ensure that the carrier protein of choice is non-self, in fact the more genetically distinct the source the greater likelihood of a larger immunogenic response.

Keyhole Limpet Hemocyanin (KLH) (Cat. No. 786-088, 786-091) is a commonly used carrier protein because it is purified from a mollusk (a gastropod) and is therefore very genetically distinct from the mammals used in antibody production. It is highly aggregated, giving it a molecular weight of 4.5×10^5 - 1.3×10^7 kDa and has a large number of available lysine groups. Common problems with using KLH as a carrier protein are a result of its large degree of aggregation, which can lead to insolubility in aqueous solutions, and the large number of coupling sites which can lead to overloading of the antigenic peptide resulting in precipitation.

Bovine Serum Albumin (BSA) (Cat. No. 786-086, 786-090) is another common carrier protein. It is smaller than KLH (67kDa), but still immunogenic. BSA is rich in lysine residues (59) of which 30-35 are available for coupling, it is highly soluble and very stable making its preparation and use very simple.

Over the years, immunological researchers have focused their attention on trying to understand the antigen recognition pathways and subsequent immunogenic responses, including antibody production. Researchers were able to demonstrate that a cationized form of BSA, produced by replacing anionic side chain carboxylic groups with aminoethylamide groups, was more immunogenic than normal BSA (1). They have shown that the amount of cationized BSA (cBSA) required for stimulation of T-cell proliferation *in-vitro* was 500 times less than normal BSA (nBSA), whereas *in-vivo* cBSA produced responses which were at least twice nBSA and lasted for longer periods of time. In addition, antibodies were produced in response to cBSA, in the

absence of adjuvants, which was not the case for nBSA.

Further research demonstrated that cBSA exhibited unique immunogenic properties as a result of alterations in the self-regulation of the immune response (2). Pretreatment with cBSA, either orally (3) or intravenously, prior to immunization with cBSA greatly enhanced the anti-BSA response; nBSA pretreatment suppressed this immune response.

The underlying mechanisms to the increased strength and duration of antibody responses are not fully understood. Researchers, however, believe that the increased positive charge ($pI > 10.5$) of cBSA gives it greater affinity for the negative membrane surface of antigen presenting cells (APCs) (4) and have shown that cBSA is taken into the cell by an adsorptive mechanism, such as receptor mediated endocytosis, as opposed to the slower fluid phase pinocytosis utilized by nBSA (5). This results in a more rapid and efficient uptake and subsequent processing of the antigen.

Interestingly, the enhanced immune response of cBSA can be extended to peptides and proteins coupled to cBSA, allowing for a greater immune response and therefore higher titer antibody production.

G-Biosciences offers HyperCarrier™ (Cat. No. 786-092, 786-096), a cationized form of BSA, as a carrier protein for the coupling of protein and peptides for antibody production. HyperCarrier™ contains BSA with various degrees of cationization, and therefore pI; as it was demonstrated that partial and fully cationized BSA elicited different enhancements on the immune response (6). It is thought that the partially cationized proteins yielded a different pattern of responsiveness due to retention of determinants necessary for the induction of T-cells, while exhibiting the enhanced immunogenicity characteristics of cationized molecules.

The HyperCarrier™ carrier protein is also available as our single use *OneQuant*™ vials to save time and money. Supplied as 8 x 2mg vials.

MATERIALS & METHOD

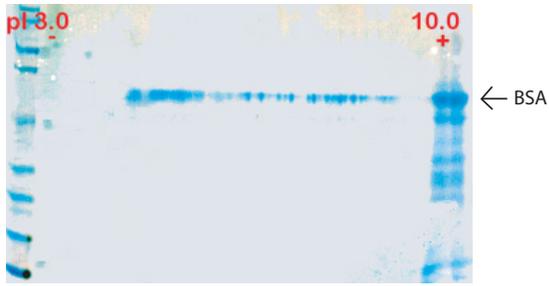
HyperCarrier™ was prepared by the coupling of ethylene diamine to the carboxyl residues using the heterobifunctional crosslinker EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride). HyperCarrier™ is supplied in two convenient sizes, a single 10mg size (Cat. No. 786-096) or our *OneQuant*™ vials (5 vials of 2mg/vial) (Cat. No. 786-092).

RESULTS AND DISCUSSION

To test the degree of cationization, 10µg HyperCarrier™ was resolved by 2D electrophoresis and the gel was stained with *RAPIDstain*™ (Cat. No. 786-31).



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The bulk of the HyperCarrier™ is highly cationized, migrating at over pI 10.0, with a small percentage migrating at various pIs ranging from normal BSA to pI >10. The ladder of protein at pI >10, is due to the high concentration of proteins, with pI >10, at the extreme end of the IPG strip, resulting in aggregation and precipitation.

ActiveHOOK™ Carrier Proteins

Immunogenic peptides are often designed with a N- or C-terminal cysteine residue that is used in the coupling of the peptide to carrier proteins through the sulfhydryl residue.

Commonly, this coupling reaction is a 2-step process. The first step couples the protein cross-linker sulfoSMCC (Cat. No. BC23) to primary amines on the carrier protein; this is followed by a desalting or dialysis step to remove excess cross-linker. The second step is the incubation of the immunogenic peptide with the activated maleimide carrier protein:cross-linker conjugate.

ActiveHOOK™ carrier proteins are preactivated; i.e. they are precoupled to the protein cross-linker. The ActiveHOOK™ carrier proteins eliminate the first step in the coupling procedure, the need to purchase protein cross-linker and the requirement for desalting or dialysis. Ultimately saving time and money!

BSA, KLH and HyperCarrier™ carrier proteins are available as ActiveHOOK™. Supplied in two convenient sizes: a 10mg vial or as 5 single-use OneQuant™ vials.

ORDERING INFORMATION

Cat. No.	Description/Size
786-096	HyperCarrier™ / 10mg
786-092	OneQuant™ HyperCarrier™ / 2mg/vial, 8 vials
786-097	ActiveHOOK™ HyperCarrier™/ 10mg
786-095	OneQuant™ ActiveHOOK™ HyperCarrier™/ 8 x 2mg
786-086	BSA (bovine serum albumin) / 10mg
786-090	OneQuant™ BSA / 2mg/vial, 8 vials
786-087	ActiveHOOK™ BSA/ 10mg
786-093	OneQuant™ ActiveHOOK™ BSA, 8 x 2mg
786-088	KLH (Keyhole Limpet Hemocyanin) / 10mg
786-091	OneQuant™ KLH / 2mg/vial, 8 vials
786-089	ActiveHOOK™ KLH/ 10mg
786-094	OneQuant™ ActiveHOOK™ KLH/ 8 x 2mg

REFERENCES

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