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THE L.E.A.P.S. APPROACH TO VACCINE DEVELOPMENT

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1. ABSTRACT

The Ligand Epitope Antigen Presentation System (L.E.A.P.S.™) approach to vaccine development utilizes immune peptides to promote the immunogenicity and influence the type of immune response generated towards epitopes in peptides which may be too small to elicit an immune response. The covalent attachment of these immune peptides to the antigenic peptide promotes the interaction of the epitope with T cells (T cell binding ligand (TCBL)) or antigen presenting cells (immune cell binding ligand (ICBL)) and ultimately promotes binding with the T cell receptor on CD4 or CD8 T cells. The "J" ICBL/TCBL peptide derived from the beta-2-microglobulin chain of MHC I molecules promotes Th1 type responses to the antigenic peptide while the "G" ICBL/TCBL peptide derived from the beta chain of MHC II molecules promotes Th2 types of responses. The efficacy of this approach has been demonstrated by characterization of the immune responses to L.E.A.P.S. vaccines and by elicitation of protection from infectious challenge with herpes simplex virus and other pathogens. The protection studies show that the L.E.A.P.S. approach allows customization of the immune response appropriate for inducing protection from disease. The theory, background, examples and studies of the mechanism of action of the L.E.A.P.S. vaccines will be discussed.

2. INTRODUCTION

The Ligand Epitope Antigen Presentation System (L.E.A.P.S.™) is a technology for converting small peptides into immunogens while steering the resulting immune response to either a predominantly Th1 or Th2 type or mixed response. The L.E.A.P.S technology covalently attaches small immune peptides to another peptide to promote the interaction of its epitope with T cells (T cell binding ligand (TCBL)) or antigen presenting cells (immune cell binding ligand (ICBL)) and ultimately promotes binding with the T cell receptor on T cells (1,2).

3. BACKGROUND

The most successful ICBL/TCBLs have been the **J peptide**, derived from the human beta-2-microglobulin chain of the MHC I molecule, (3) and the **G peptide**, derived from the second domain of the beta chain of the human MHC II molecule (4,5). A list of the ICBL/TCBLs and some of the peptides incorporated into heteroconjugates is presented in Table 1. These peptides were originally chosen because they were considered to be from those portions of the MHC molecular complexes that interact with CD8 or CD4 molecules. We subsequently showed that immunogens constructed with the J ICBL/TCBL direct immune responses to the attached peptide epitope toward Th1 type of immune responses and the G ICBL/TCBL directs the response toward a Th2 type of response. Heteroconjugate vaccines constructed with the peptide J as the ICBL/TCBL have elicited the best protection from infectious challenge and exhibited, if detectable, predominantly IgG2a antibody (2,6,7,8). Heteroconjugate vaccines constructed with the peptide G as the ICBL/TCBL are best at enhancing antibody production, predominantly IgG1 (2,8,9,10). An additional ICBL/TCBL, the **F peptide**, is from IL-1 beta, and it activates a more generalized immune response to its heteroconjugate.

The activity of the G ICBL/TCBL can be attributed to its ability to interact with CD4. The ability of the G ICBL/TCBL to bind to CD4 molecules was shown by peptide competition studies and binding was lost upon site directed mutagenesis of select amino acid residues in

Table 1. Peptides Sequences Used In Preparation Of L.E.A.P.S.™ Constructs

TCBL	Peptide sequence	Molecular source	Reference
G	NGQEEKAGVVSTGLI	MHC-II beta 2 (135-149)	1,2,4,5,6,8,9
J	DLLKNGERIEKVE	beta-2 microglobulin (aa 38-50)	1,2,3,6,7,8,9
IL-1beta	VQGEESNDK	IL1beta (163-171)	1,2,10
Control			
L	LHGPEILDVPST	Laminin/Fibronectin (not applicable)	1,2,8
Antigenic peptides			
HGP-30	YSVHQRIDVKDTKEALEKIEEEQNKSKKKA(aa 85-115)	HIV-1 B p17	9,10
m-HGP-30	ATLYSVHQRIDVKDTKEALEKIEEEQN (aa 82-112	HIV-1 B p17	10
M	DQVHFQPLPPAVVKLSDAL(aa350-369)	M. tuberculosis 38 kDa	2
H1	LYRTFAGNPRA(aa 322-332)	HSV 1 ICP27	6,8
gB1	SSIEFARL# (aa 498-505)	HSV 1 gB	8
gD1	SLKMADPNRFRGKDLP # (8-23)	HSV 1 Glycoprotein D	6,7,8
# common to HSV 1 & 2			

the MHC II beta-chain region domain of this peptide (4,5). The CD4 molecule is present on specific subclasses of T cells, macrophages, and dendritic cells (DC) (see review 11 by Bowers *et al*). CD4 is both a co-receptor with the TCR for antigen recognition as well as being a co-stimulator molecule on T cells (11,12,13). The role and function(s) of CD4, and perhaps also CD8, on macrophages and DCs are not yet clear.

The J ICBL/TCBL peptide sequence is derived from a region of the beta-2-microglobulin molecule component of the MHC I complex. Beta-2- microglobulin (B2M) is a highly conserved protein across many different mammalian species (14). The peptide region which contains the J ICBL/TCBL is within one of two dominant B2M epitopes and is accessible for intermolecular interactions since it is on the surface of the MHC I molecular complex. This region, centered around the arginine 45 of B2M, can be recognized by a monoclonal antibody (referred to as anti-SWH.5) (3). The immunological importance of this region is indicated by the following: a polyclonal antibody to this region of B2M will block allogeneic T cell responses in a mixed lymphocyte reaction (14); and addition of murine or human B2M or proteolytically cleaved B2M can enhance cytotoxic T cell activity and promote CD4 T cell proliferative responses (15,16). Since the development of the J ICBL/TCBL, others have shown that covalent attachment of the entire B2M protein, either chemically or as genetically engineering fusion proteins, to an epitope can enhance antigen presentation of the epitope (17,18,19). Use of the entire B2M protein or other peptide regions in a vaccine heteroconjugate may also promote other interactions which may or may not improve the immunogenicity of the epitope. For example, the ability of B2M to bind to alpha-2-macroglobulin can be attributed to a peptide which overlaps the last two residues (aa 49,aa 50) of the J ICBL/TCBL (20) but it is unlikely that this will mediate an interaction like that observed with the J peptide. Most importantly, the J ICBL/TCBL contains the functions that promote immunogenicity and steer the immune response without other, potentially irrelevant or detrimental functions.

It is interesting to speculate that the L.E.A.P.S conjugates are similar to a hypothetical benign superantigen which binds initially through the ICBL/TCBL to an MHC molecule and then directs the epitope towards binding to the T cell receptor. Unlike a typical superantigen which non-specifically activate numerous T cells, the L.E.A.P.S. conjugates would activate only a limited number of antigen specific cells to elicit appropriate immune response (21).

4. COMPARSION WITH OTHER EPITOPE DELIVERY TECHNOLOGIES

Although an epitope is sufficient to be recognized by antibody or an MHC molecule, it is often too small to cross-link immunoglobulin on the B cell or lacks the means for interacting with antigen presenting cells for display to the T cell receptor (TCR) on T cells. The L.E.A.P.S. approach to converting an epitope into an immunogen is different from other approaches. The classic means for conversion of an epitope into an immunogen is its attachment to a large carrier protein. These large proteins include keyhole limpet hemocyanin (KLH) and ovalbumin, used in the laboratory; and more recently Heat Shock Proteins (22), under testing for human use; diphtheria toxoid (23), which is used as part of the HIB and pneumococcus conjugate vaccines on the market; and even larger units such as virus-like particles (VLP) (24). The purpose of the carrier element is to facilitate phagocytic or pinocytotic uptake by a dendritic or other type of antigen presenting cells (APCs), promote appropriate proteolytic processing, and intracellular transport to the appropriate compartment for binding to MHC molecules and subsequent presentation to T cells. In general, immunogens which utilize large proteins as carriers generate Th2 type responses, with an emphasis on antibody production. Another potential detrimental side effect with the use of the large protein carrier approach is the generation of a significant immune response against the carrier protein molecule (25). In contrast to the large protein carriers, the entire heteroconjugate L.E.A.P.S. vaccine molecule may be as small as 26 amino acids and the ICBL/TCBL does not elicit a detectable immune response. In addition, the J ICBL/TCBL elicits a Th1 type of immune response to the covalently linked epitope (1,2,6,7,8,9,10).

The J and G ICBL/TCBL peptides differ from other epitope enhancers in the manner by which they promote the immunogenicity of an epitope. There is no evidence to suggest that the L.E.A.P.S. vaccines act as an MHC restricted helper epitope in a heteroconjugate in order

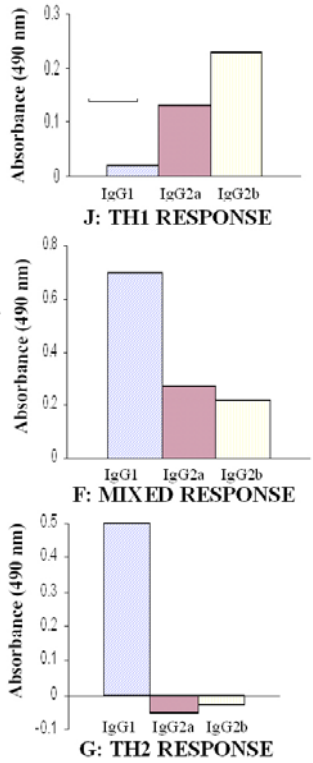

Figure 1. Influence of the covalently attached TCBL/ICBL within the L.E.A.P.S. heteroconjugate on the induction and isotype of the antibody from animals immunized and challenged with a Mycobacterium tuberculosis epitope. Peptide heteroconjugates of the 38.G epitope (peptide M) of the 38 kDa protein (65) from Mycobacterium tuberculosis were prepared with the Th1 directing TCBL peptide J from beta-2-microglobulin, the Th2 TCBL was the peptide G from the second domain of the MHC II beta chain or a the F peptide from Interleukin-1 beta (66) by the method of Robey (66). Balb/c mice were immunized on days 1, 15, and 49 with peptide conjugates in phosphate buffered saline emulsified in incomplete Freunds Adjuvant. Test bleedings were taken from anaesthetized mice from the retro-orbital sinus taken on day 63 and there was no specific antibody response. The mice were boosted with peptide M conjugated to KLH and 14 days later test bleedings were taken. Sera were evaluated at a screening dilution of 1:200. Figure adapted from Zimmerman *et al* (2).

to facilitate interaction of antigenic epitopes with T cells, such as studied by Francis (26), Good (27) or Milich (28), or as an enhanced universal (Pan) T cell helper epitope (Padre®, 29). Nor does the L.E.A.P.S. approach resemble the Ii-Key peptide conjugate approach which is suggested to open the peptide binding site of MHC II molecules to promote the interaction and presentation of an antigenic epitope (30). The sequence of the J and G ICBL/TCBL and their lack of extensive portions or regions of basic amino acids also argues against an action similar to the SynB vectors which enhance the transport of SynB conjugates across the cell membranes (31,32,33), or cell entry via the beta defensin route (34). The lack of a lipid or highly hydrophobic component argues against its acting as a hydrophobic unit to facilitate insertion and initial anchoring into the cell membrane (35), such as used in the Theradigm® approach (36). Although these approaches elicit immune responses in well established *in vitro* assays such as immune cell proliferation, CTL killing, cytokine production, or immunogen binding to cells, the relevance of these immune response to protection from infectious or tumor challenge may not have been established.

5. REVIEW OF RESULTS WITH L.E.A.P.S. VACCINES

5.1. Immune response to epitope

Despite their small size, the J and G ICBL/TCBL act as immunological carriers and convert a covalently attached small peptide epitope into an immunogen capable of eliciting an immune response. The ability of the J and G ICBL/TCBL to bind to CD8 and CD4 molecules appears to be responsible for their ability to convert epitopes into immunogens. These interactions are likely to direct and promote the binding of a peptide epitope to specific antigen presenting cells, such as dendritic cells, or T cells. A T cell response is initiated, with or without an antibody response. The response is of either a Th1 or a Th2 type depending upon whether the J or the G ICBL/TCBL is attached to the peptide.

Initial studies with the 38.G epitope of the 38kDa protein from Mycobacteria tuberculosis demonstrated the potential of the L.E.A.P.S. technology to promote and steer the type of antibody production to the epitope to either a Th1 or a Th2 type response (2). Activation of T cells by heteroconjugates of the 38.G epitope with the J or the G ICBL/TCBL was indicated by the initial induction of a heteroclictic response (non-specific increase in background antibody production) followed by a strong and more rapid secondary Th1 or Th2 type of immune response to challenge with a conventional 38.G-KLH immunogen. This is clearly illustrated in Figure 1 (adapted from Zimmerman *et al* (2)) which demonstrates that the isotype of the antibodies to the same epitope is strongly influenced by the ICBL/TCBL used in the preparation of the heteroconjugate. The J-38.G heteroconjugate elicited the production of Th1 associated IgG isotypes while the G-38.G heteroconjugate elicited Th2 associated IgG isotypes. The F-38.G heteroconjugate activates immune responses differently than the others and elicited a mixed response which was predominantly Th2 in nature. In contrast to the L.E.A.P.S. heteroconjugate vaccines, only a Th2 type of response could be elicited by the 38.G-KLH immunogen. These studies indicated that the L.E.A.P.S. vaccine activated T cells and directed the nature of the subsequent immune response.

Although the F ICBL/TCBL has not been developed as extensively as the J and the G ICBL/TCBLs, L.E.A.P.S. vaccines were developed using the HGP-30 peptide from the HIV p17 gag protein with the F, as well as the J and the G ICBL/TCBLs (9). This L.E.A.P.S. F-HGP30 vaccine elicited proliferative, CTL and IFN-gamma

Table 2. Reactivity of antisera of mice immunized with various m-HGP-30 immunogens

Immunogen	Day of TB	A490 at Screening Dilution		
		m-HGP-30	Peptide G	KLH
H-KLH	47	2.099	0.039	2.482
	120	2.392	0.053	2.999
GH	47	1.167	0.023	0.189
	120	3.248	0.034	0.576
Peptide G	47	0.002	0.013	0.111
	120	0.104	0.043	0.094
G-KLH	47	0.009	1.986	2.463
	120	0.013	2.745	3.276

responses and these responses were strongly influenced (enhanced) by pretreatment with the Flt3 ligand molecule (10), a growth factor known to enhance cellular immune responses (see the following recent reviews 37,38,39) before L.E.A.P.S. vaccine immunization.

Subsequent studies demonstrated the ability of the L.E.A.P.S. technology without use of Flt3 ligand treatment to promote protection from infectious challenge and specific immune responses to epitopes from the herpes simplex virus ICP27 protein (6) and glycoproteins D (gD1) (7) and B (gB1) (8). These studies will be described further in a subsequent article in this series.

5.2. Protection and other *in vivo* studies

The L.E.A.P.S. heteroconjugate vaccines are likely to work by a "prime-boost" mechanism in which they prime the immune system by activating T cells such that subsequent challenge provides the boost that completes the immune response and promotes Th1 antibody, DTH-like, IFN-gamma, and T cytotoxic cell responses at the time that they are needed. This is different from that elicited by a classical epitope-protein carrier immunogen and several other peptide enhancement systems which are more focused on antibody production. The ability of L.E.A.P.S. vaccines to initiate T cell responses could be discerned by the following: by the presence of DTH responses (7,8), a more rapid, secondary type of antibody response upon challenge with conventional antigen or infection (2,7,8), and by elicitation of immunoprotection from challenge using an immunogen which does not generate antibodies (6).

The first indication that L.E.A.P.S. vaccines elicit protection from infectious challenge was with the JH1 vaccine. The JH1 vaccine incorporates a known CTL protective epitope from the herpes simplex virus type 1 protein ICP27. This vaccine elicited protection and DTH responses but not antibody (6). Immunization with this heteroconjugate or other vaccines with the ICP27 protein do not elicit antibodies and even if they did, the antibodies would not be protective since the ICP27 protein is not part of the virion and is inaccessible to antibody. These studies provided the proof of principal that L.E.A.P.S. vaccines elicit T cell responses and they are protective from lethal infectious challenge.

Protection from HSV-1 challenge was also demonstrated with J L.E.A.P.S. vaccines incorporating another epitope from ICP27 and epitopes from glycoproteins B and D (6,7,8). Antibody to the B cell epitope(s) within the 'SSIEFARL' CTL epitope of the HSV glycoprotein B (JgB) and a 16 amino acid peptide from the N-terminus of glycoprotein D (JgD) were only detected after infection, following exposure to the entire protein (7, 8). Although L.E.A.P.S. vaccines with the G-ICBL did not elicit protection against HSV challenge, the G-L.E.A.P.S. vaccine incorporating the glycoprotein D peptide (GgD1) was sufficient to elicit a Th2-related antibody response without an antigenic boost.

The ability of the J ICBL/TCBL to promote Th1 responses may also be useful to counteract or reverse some autoimmune responses that are induced by Th2 responses. Other unpublished work indicates that L.E.A.P.S. vaccines can promote protection against experimental autoimmune myocarditis in an animal model (Rose unpublished results).

The ability of the J- L.E.A.P.S. vaccines to initiate Th1 responses allowed the development of protective vaccines. The generation of antibody, the usual marker of vaccine efficacy, did not necessarily correlate with protection. This was illustrated in two ways: the GgD1 vaccine elicited antibody but not protection, and stronger antibody responses were observed in those mice with greater symptoms (presence of a larger antigenic load). These examples provide another indication that *ex vivo* assays of immunepotentiation for a vaccine may not correlate with elicitation of protection. Other assays using defined and potentially restricted or homogenous cell populations do not address all the complex interactions that occur in the intact host and all too often results in a discordance between *in vivo* and *ex vivo* findings (see for example the following recent reviews 40,41,42).

6. MECHANISTIC STUDIES OF L.E.A.P.S. VACCINES

6.1. Lack of antibody response to the ICBL

Unlike conventional carrier proteins, little or no antibody/ immune response is initiated against the ICBL or TCBL peptides of the L.E.A.P.S. vaccine. This is demonstrated for the G ICBL/TCBL in Table 2. In this experiment, the antibody response of Balb/c mice to the protein carrier, KLH, is compared to the response to the G ICBL/TCBL after immunization with the G ICBL/TCBL by itself, or a heteroconjugate which includes the HGP-30 peptide (H) from the HIV p17 gag protein conjugated to the G ICBL/TCBL or KLH, or a heteroconjugate of the G peptide and KLH (9). The ELISA data in this experiment indicates that strong and specific antibody responses were elicited by heteroconjugates with the H peptide and either

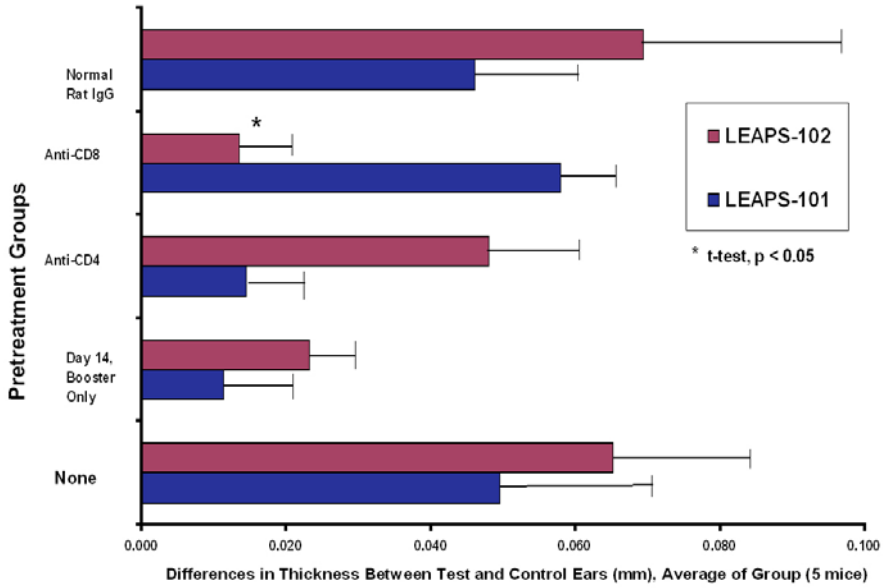

Figure 2. Immune components involved in the induction of immune responses to the G-HGP-30 (LEAPS 101) or the J-HGP-30 (LEAPS 102) vaccines. The effects of monoclonal antibody ablation prior (administered daily on day -5 to day 0) to immunization with the G-HGP-30 (LEAPS 101) or the J-HGP-30 (LEAPS 102) vaccines were evaluated by testing the residual DTH immune responses. DTH responses to HGP-30 peptide challenge in the ear were evaluated 28 days after immunization (10) [for more details on L.E.A.P.S. vaccines, immunization, test bleeding or ELISA assays see Zimmerman *et al* (9)].

KLH or the G ICBL/TCBL. Not surprisingly, the antibody titer to KLH was stronger than the response to the HGP-30 epitope. Such a response could be detrimental, especially if this protein carrier were to be used in other vaccines intended for human use. In contrast, the response to the G peptide in the heteroconjugate with the H peptide epitope or the peptide alone was borderline or non-existent. No responses were obtained to the J ICBL/TCBL in similar studies. Although the G peptide can elicit a response when attached to KLH, the lack of response to the G and J L.E.A.P.S. ICBL/TCBL peptides present in a vaccine is most likely due to the nature of the interaction of the L.E.A.P.S. peptide with the CD4 or CD8 co-receptor on DCs or T cells and the consequences of the interaction.

6.2. Identification of relevant immune responses

The immune components and responses relevant to L.E.A.P.S. vaccine initiation and delivery of immunity and protection were identified by the consequences of its ablation or neutralization by antibody. In the first experiment, the involvement of interferon gamma and different immune cell populations in generation of a DTH response to the J-LEAPS- HGP-30 peptide heteroconjugate (referred to as LEAPS 102) of a G-LEAPS-HGP-30 heteroconjugate (referred to as LEAPS 101) were determined. DTH responses are one indication of immune responses relevant to protection from many types of infections. Groups of Balb/c mice were treated prior to immunization with different monoclonal antibodies specific for CD4, CD8, IFN-gamma or as a control, a rat immunoglobulin. The results shown in Figure 2 demonstrate that the DTH response required the presence of CD8 cells during the induction phase since anti-CD8 significantly reduced the response. Treatment with antibody to CD4 or IFN-gamma prior to immunization did not significantly change the response. This is consistent with the interaction of the J ICBL/TCBL with CD8 molecules.

In a similar ablation experiment, antibody ablation of CD4 cells prior to immunization reduced the DTH and antibody response to the G-HGP30. As Figure 3 clearly demonstrates, CD4, and to a lesser extent CD8 cells, are important for antibody responses to the HGP-30 peptide since antibody ablation of these cells prevents or reduces the Th2-associated IgG1 antibody production. Control antibodies had no effect on immune responses.

Antibody ablation and neutralization were also used to identify the immune components important for protective responses to the HSV-1 JgD vaccine (7). Similar to the LEAPS 102 vaccine, ablation of CD8 cells prior to immunization eliminated protection while ablation of CD4 cells or interferon gamma either before or after immunization, but prior to challenge, also eliminated the protection. These results indicate that in the Balb/c mouse, CD8 cells are required only for initiation of the response to JgD, but interferon gamma and CD4 cells are essential for both initiation and delivery of the protection.

7. MODE OF ACTION

The original concept for the mechanism of action of the G and J peptides in L.E.A.P.S. heteroconjugate vaccines was that of a T cell binding ligand (TCBL). Binding of the G or J peptides to the CD4 or CD8 molecules on the T cell would deliver and anchor a covalently linked peptide epitope near to the TCR and allow it to "fall" into the antigen binding site to stimulate activation of the cell and at the same time, deliver a co-

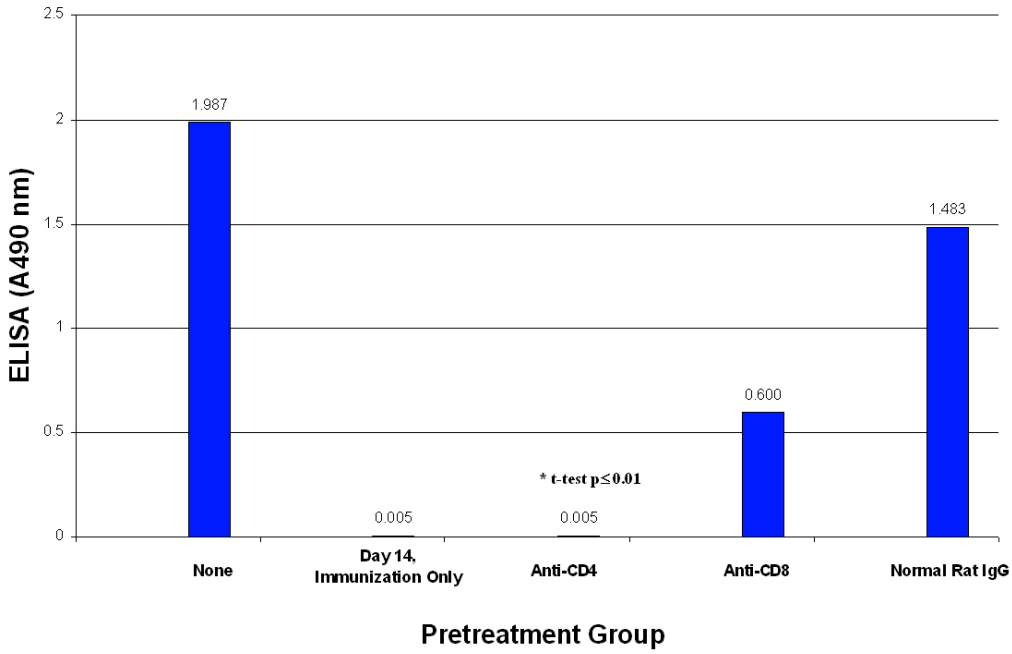

Figure 3. Immune components involved in the induction of immune responses to the G-HGP-30 (LEAPS 101) vaccine. Mice received monoclonal antibody ablation prior (administered daily on day -5 to day 0) to immunization with LEAPS 101. On day 28, blood was obtained and serum antibody to HGP-30 was evaluated at a screening dilution of 1:200 by ELISA [for more details on L.E.A.P.S. vaccines, immunization, test bleeding or ELISA assays see Zimmerman *et al* (9) and see Figure 2].

stimulatory signal to the T cell. In an alternate model the G and J peptides are also immune cell binding ligands (ICBL). This model proposes that the L.E.A.P.S. peptides facilitate the binding of the antigenic peptide to CD4 or CD8 molecules on the surface of specific subtypes of antigen presenting cells (APC), such as the specialized professional APC or dendritic cells (DC), which can then efficiently present the epitope to T cells. In this manner, internalization and processing of the immunogen would not be necessary. Once attached to the CD4 or CD8 molecule on APCs, the epitope portion of the vaccine may be able to find the epitope binding cleft of a nearby MHC molecule to be presented to a T cell. This would be similar to the peptide epitope loading of MHC I molecules (without intracellular processing) which is used to generate target cells for *in vitro* identification of epitopes for specific cytotoxic T cell killing.

The ability of the J and G L.E.A.P.S. ICBL/TCBLs to direct the subsequent immune response to a Th1 or Th2 response is most likely due to their binding to different target cells, either T cells, DCs, or macrophages, as defined by the expression of either CD8 or CD4 and other CD molecules on their cell surface. The subsequent immunological response would be determined by the function of the different target cells, the cytokines that they produce and the type of T cell or other type of cell with which that target cell interacts (43,44,45). As a T cell binding ligand (TCBL), the J and G peptides should interact with different T cell populations. Although there is some cross over between the cytokines secreted and functions attributed to CD4 and CD8 T cells, these cells have different functions. As an immune cell binding ligand, (ICBL), the interaction of the G or J peptide with different types of DCs, as determined by the expression of either CD4 or CD8, is likely to produce different cytokines and promote different immunological outcomes. For example, CD8 expressing mouse DCs produce IL-12 and IL-18 in response to challenge (46,47,48). Stimulation of IL-12 and IL-18 production by DCs and other cells early in the response will promote the production of interferon gamma which will solidify the nature of the response as a Th1-type of response (50). The response of CD4 expressing antigen presenting cells is different (49). These initial interactions with the immune system are very important for determination of the direction that the subsequent immune response will follow. It is therefore likely that the directed binding of a J-L.E.A.P.S. peptide heteroconjugate vaccine to a CD8 bearing T cell or DC steers the subsequent response to a Th1 response. Binding of a G-L.E.A.P.S. peptide heteroconjugate vaccine to a CD4 expressing Th2 prone T cell or DC, may also define the subsequent direction of the response or allow the response to follow a default Th2 direction. More research is necessary to determine the mechanisms by which the J and G ICBL/TCBLs steer the immune response to a L.E.A.P.S. heteroconjugate vaccine.

The covalent attachment of the L.E.A.P.S. ICBL/TCBL to the epitope is required since a mixture of the peptides does not seem to be effective. This suggests that the ICBL/TCBL is a delivery vehicle to CD4 or CD8 molecules, or a concerted signal or cross-linking of receptors is required for a response. One possible mechanism for L.E.A.P.S. vaccine activation of immune responses is the delivery of a co-stimulatory signal to the APC or the T cell

through the binding of the G or the J peptides to CD4 or CD8 molecules. This signal may either activate the DC or T cell targets or eliminate a suppressive reaction to the immunogen. We favor the concept that the L.E.A.P.S. J and G ICBL/TCBLs deliver an activating signal to T cells or DCs bearing CD4 or CD8 molecules. This signal may promote more effective antigen presentation or the production of appropriate cytokines to activate and steer the immune response to a Th1 or Th2 type of response. The cytoplasmic tail of the CD4 and CD8 molecules interact with the p56(lck) kinase,(51,52) which initiates a cascade of phosphorylation and dephosphorylation events to modulate the cell's response to a specific antigen (53,54,55,56) but how antigen specificity is maintained is still a mystery**.**

Studies with the murine analogue of the G peptide ICBL/TCBL by Konig *et al* (2,57,58) demonstrated an immunomodulatory activity for the peptide. Initially, Konig suggested that the peptide enhances immune responses by preventing suppression or tolerization by blocking the activation-induced cell death of T cells (57). Later, they showed that the peptide has direct effects on the T cell which promotes mobilization of intracellular calcium ion, activation of cyclic AMP phosphodiesterases and other biochemical actions which are important for full activation of antigen–stimulated CD4 T cells (58). These studies suggest that the G peptide ICBL/TCBL component of a heteroconjugate L.E.A.P.S. vaccine may provide an activation signal to the cell through their interaction with the CD4 associated p56(lck) kinase. The influence of the CD4 associated p56(lck) kinase on the cell is also evident for HIV infection. Binding of the gp120 of HIV to CD4 can promote anergy in naïve T cells and the tyrosine kinase activity of the p56lck is detrimental to the replication of the virus (59,60).

As an outgrowth of the development of L.E.A.P.S. vaccines, we demonstrated that an analogue of the G ICBL/TCBL, CEL-1000 (61,62), in which the N terminus is changed from asparagine to aspartic acid, activates innate responses which promote Th1 responses, perhaps by mechanisms similar to those described by Konig (57,58). It is interesting that the CEL-1000 peptide alone activates an innate response that promotes Th1 immune responses whereas heteroconjugates in which an epitope in covalently attached to the related G peptide produce Th2 type responses.

The interaction of the J-ICBL with the CD8 molecule is also likely to elicit a signal to the target cell. Binding to the CD8 molecule also activates the p56(lck) on CD8 T cells and is important for production of perforin and IFN gamma (63). In addition, the CD8 molecule may also be important for promoting the production of IL-12, IFN-gamma and other cytokines by DCs (45,46,64).

8. PERSPECTIVE

Another article in this series will describe vaccines prepared utilizing the L.E.A.P.S. technology for protection or treatment of infections of herpes simplex virus (HSV). Other L.E.A.P.S vaccines have, or are being developed for Human Immunodeficiency Virus (8), Mycobacteria tuberculosis (2), and experimental autoimmune myocarditis (Rose unpublished observations). In the latter case, the L.E.A.P.S. technology is being utilized to alter the immune response and outcome of an autoimmune disease, autoimmune myocarditis, by shifting a Th2 response to a Th1 type of immune response.

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