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LIGAND EPITOPE ANTIGEN PRESENTATION SYSTEM VACCINES AGAINST HERPES SIMPLEX VIRUS

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1. ABSTRACT

The Ligand Epitope Antigen Presentation System (L.E.A.P.S.™) approach to vaccine development allowed construction of immunogens from defined T cell epitopes from herpes simplex virus (HSV) proteins that conferred protection against lethal challenge by the virus. This technology utilizes specific peptides which bind to CD4, CD8 or other proteins on the surface of T cells (T cell binding ligand (TCBL)), macrophage and dendritic cells (immune cell binding ligand (ICBL)) to promote the immunogenicity of an epitope, activate T cell and other protective responses, and direct the immune response to either a Th1 or a Th2 type of response. The J TCBL/ICBL is a peptide from beta-2-microglobulin which binds to the CD8 protein and promotes Th1 responses and the G TCBL/ICBL is a peptide from the beta chain of MHC II molecules that binds to the CD4 protein and promotes Th2 responses. Epitopes from the ICP27 (H1, H2), glycoprotein B (gB) and glycoprotein D (gD) proteins of HSV-1 were attached to either the J TCBL/ICBL or the G TCBL/ICBL. The JH1, JH2, JgB and JgD vaccines elicited DTH responses without antibody but conferred protection upon lethal challenge. Th1 related antibody was produced after challenge of the JgB and JgD immunized mice. Immunization with the GH1, GgB or GgD vaccines did not yield protection. The GgB and GgD produced Th2 related antibodies upon virus challenge. Initiation of the immune response by the JgD vaccine was dependent on functional CD4, CD8 expressing cells and interferon gamma and delivery of protection was dependent upon CD4 and interferon gamma. The L.E.A.P.S.™ HSV vaccines appear to elicit the appropriate immune responses for protection and further work is being performed to develop the JgD vaccine for human use.

2. INTRODUCTION

The goal of every vaccine is to elicit long term protection from infection; however, the nature of the protective immune response is different for different infectious agents. For some viruses, toxins and most bacteria, a neutralizing antibody (generated by a Th1 or a Th2 helper T cell response) is sufficient to prevent disease by blocking dissemination in the blood, interaction with its target cell, or by opsonization. Antibody can be sufficient to resolve infections by cytolytic viruses because the virus kills the cellular factory and the antibody neutralizes the cell-free virus. In contrast, antibody is not sufficient to protect against many enveloped and non-cytolytic viruses (1), herpes viruses, (2,3) human papillomavirus, HIV (4,5), intracellular bacteria (salmonella, mycobacteria, etc.) (6) and, fungi (7). These infections require cell mediated (Th1) immune responses to activate macrophages and T cells to prevent serious disease (8). Antibody, neutralizing or otherwise, may not be necessary and usually is not sufficient for protection against a non-cytolytic virus infection; since the cellular factory will continue to produce virus despite control of the cell-free virus. For example, the importance of cell mediated immunity for protection and defense against HSV infection, both primary and recurrent, is demonstrated by the severity of symptoms that occur in neonates, who lack a mature cell mediated immune response, or immunosuppressed individuals who were previously infected with HSV and have antibody to the virus (9).

The L.E.A.P.S. (ligand epitope antigen presentation system) vaccine technology was developed to allow the design of vaccines that activate T cells to elicit the appropriate protective response to a specific antigenic target. This technology utilizes specific peptides which bind to CD4, CD8 or other proteins on the surface of T cells (T cell binding ligand (TCBL)), macrophage and dendritic cells (immune cell binding ligand (ICBL)) to promote the immunogenicity of an epitope, activate T cell and other protective responses, and direct the immune response to either a Th1 or a Th2 type of response. The J TCBL/ICBL is a peptide from beta-2-microglobulin which binds to the CD8 protein and promotes Th1 responses and the G TCBL/ICBL is a peptide from the beta chain of MHC II molecules that binds to the CD4 protein and promotes Th2 responses (Table 1). A heteroconjugate peptide vaccine is synthesized by combining an ICBL/TCBL with a

Table 1. Ligand Epitope Antigen Presentation System And Epitope Peptide Sequences

Peptide		Sequence	Molecular source	Reference
ICBL'S	G	NGQEEKAGVVSTGLI	MHC-II β2 (aa135-149)	38,39
	J	DLLKNGERIEKVE	β-2 microglobulin (aa35-50)	40
EPITOPES	H	LYRTFAGNPRA	HSV-1 ICP27	12
	H2	DYATLGVGV	HSV-1 ICP27	12
	gB	SSIEFARL	HSV-1 Glycoprotein B	16
	gD	SLKMADPNRFRGKDLP	HSV-1 Glycoprotein D	13

T and/or B cell epitope to convert even the smallest peptide epitopes (8 amino acids) into immunogens. (10, 11, 12, 13, 14, 40).

The first study using the L.E.A.P.S. technology evaluated the ability of heteroconjugate vaccines utilizing the M peptide of *M. tuberculosis* attached to one of several L.E.A.P.S. ICBL/TCBL peptides to elicit antibody in mice (11). The initial results obtained upon immunization of mice with the L.E.A.P.S. heteroconjugate vaccines were disheartening since a specific antibody response could not be detected. However, challenge of the immunized mice with a classical antigen (M-KLH) resulted in a secondary antibody response indicative of the presence of vaccine-stimulated T cells. The vaccine-stimulated T cells appeared to provide the cytokine help necessary to promote immunoglobulin class switch. A more interesting finding was that the predominant antibody subclass response was determined by the ICBL/TCBL peptide incorporated into the heteroconjugate vaccine. The J-ICBL peptide in the J-M heteroconjugate appeared to direct the immune response to produce antibody to the M protein of the IgG2a subtype, characteristic of a Th1 response, while the G-M peptide generated IgG1 subtype antibodies, indicative of a Th2 response after the M-KLH challenge. These results are in contrast to a more limited Th2 response following immunization and challenge with M-KLH. The data suggests that the L.E.A.P.S. heteroconjugate primes the immune response to the antigen by activating T cells, which upon challenge with antigen (M-KLH) promoted (boosted) the more complete immune response, including antibody. The actual mechanism by which the J and G ICBL/TCBL peptides direct the immune response is not known.

The proof of the principle and demonstration of efficacy for L.E.A.P.S. vaccines was provided with the development of L.E.A.P.S. anti-herpes simplex virus (HSV) vaccines. HSV is a common oral pathogen and sexually transmitted disease which can cause serious infections of the eye, encephalitic infection of the brain or disseminated infection of the neonate, AIDS patient or otherwise immunocompromised individuals. Control of HSV infections requires T cell immune responses. Well established mouse models of human HSV disease allow determination of vaccine protection from disease or death as well as an evaluation of the nature of the immune response. For HSV-1 infection of the mouse, protective T cell immunity is directed towards specific peptides from several viral proteins including ICP27 (infected cell protein 27) (12,15), glycoprotein B (gB) (16), and glycoprotein D (13,17,18). By themselves, the peptides containing these epitopes are too small to be effective immunogens and cannot elicit protective responses.

3. L.E.A.P.S. VACCINES WITH H1 AND H2 EPITOPES FROM THE ICP27 PROTEIN OF HSV-1

The first trial of a L.E.A.P.S. anti-HSV vaccine was attempted with a peptide (H1) from ICP27. The ICP27 protein contains two CTL epitopes (aa 322-332 and 448-456) which can elicit protective immune responses (15) in H2d (Balb/C), but not H-2k or H-2b mice. For this initial trial, the aa 322-332 epitope (H1) was incorporated into L.E.A.P.S. heteroconjugates with the J (JH-1) and G (GH-1) ICBL/TCBLs. Later, heteroconjugates with the aa 448-456 peptide epitope were prepared (JH-2, GH-2).

Epitopes from ICP27 were chosen because ICP27 is an immediate early, nuclear protein of HSV which elicits a prominent T cell protective response but does not elicit a detectable antibody response and antibody would not be protective. Rouse et al. showed that up to 25% of a Balb/C mouse T cell response is directed towards ICP27 during natural infection (19). Interestingly, immunization of mice with a vaccinia virus containing the ICP27 gene protected the mice from lethal challenge. The immune response was shown to be a Th1 CD4 T cell response but there was very little antibody to ICP27 produced by the vaccination (detectable by western blot but not ELISA) (20).

In the first infectious challenge study with the L.E.A.P.S. vaccines, the JH-1 heteroconjugate elicited protection from the morbidity and mortality of an intraperitoneal challenge with HSV-1 but no antibody to ICP27 could be detected (12) (Figure 1). The mice had been immunized with the vaccine mixed with Novosome™ adjuvant on two occasions with a 14 day interval. In addition to protection, the JH1 vaccinated, unchallenged mice could mount a DTH response to UV inactivated HSV. Elicitation of protection by vaccination with the JH1 vaccine demonstrated the efficacy of the L.E.A.P.S. vaccine technology. The study also reaffirmed that activation of T cell responses are sufficient to elicit protection from HSV disease. Even if antibody were produced, it would have no potential to neutralize the virus or to elicit protection to the intracellular ICP27 protein. Most importantly, it supported the hypothesis that L.E.A.P.S. vaccines elicit T cell responses. The ability of the JH1 vaccine to elicit a Th1 response was indicated by the nature of the protection and the presence of a DTH response in the immunized animals. The corresponding GH1 heteroconjugate elicited no protection and these mice may have had a worse outcome than the unimmunized mice. This result is consistent with the establishment of a Th2 type of response to immunization with GH1. Initiation of a Th2 response by the vaccine might prevent the mouse from activating a protective Th1 response to HSV challenge and result in exacerbation of the viral disease.

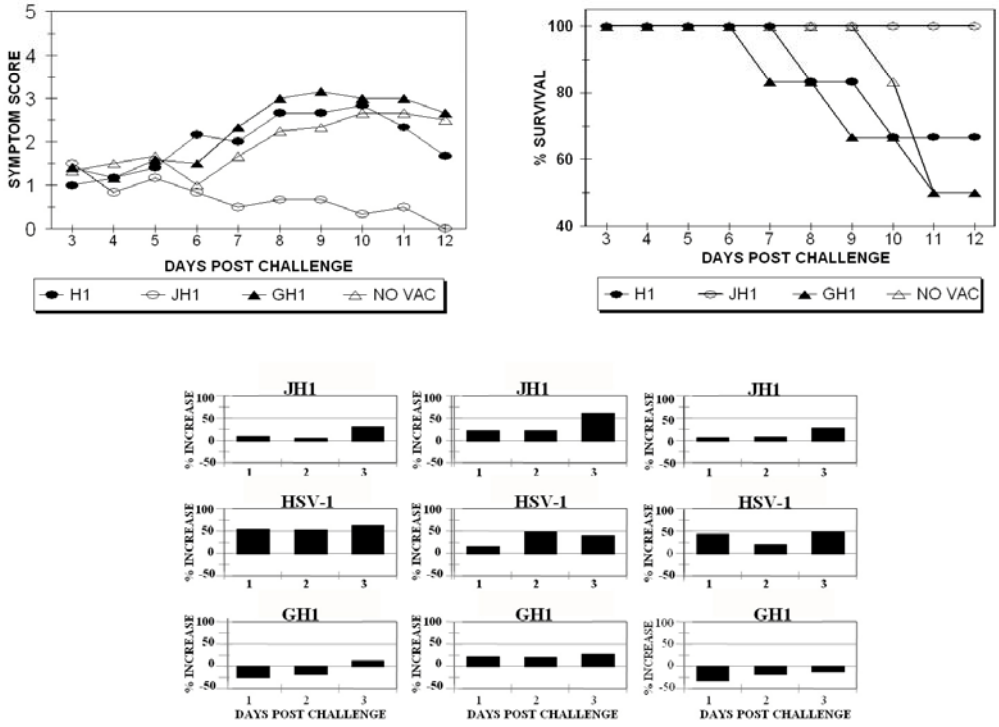

Figure 1. Immunization with JH1 protects Balb/C mice (n=7) from lethal intraperitoneal HSV-1 challenge and promotes DTH responses to virus. Mice were unimmunized or immunized twice with JH1, GH1, or the H1 peptide in Novosome adjuvant with a 14 day interval. After an additional 14 day interval, the mice received an intraperitoneal challenge with the H129 strain of HSV-1. Symptoms were scored as 0: no symptoms, 1: non-specific signs; 2: mild swelling of the abdomen or other signs of infection; 3: swelling of abdomen, crippling or debilitation; 4: severe signs; 5: death. DTH responses were evaluated in immunized but uninfected mice as the percent increase in swelling of the ear pinna after injection of UV inactivated HSV for individual mice.

This study proved that L.E.A.P.S. vaccines could elicit protective responses and the protection was elicited through activation of specific T cell responses.

Based on the success of the JH1 vaccine, L.E.A.P.S. heteroconjugates were prepared with the H2 epitope (448-456) from ICP27. Mice immunized with the JH2 vaccine in Novosomes had less disease and greater survival than the unimmunized controls (Figure 2). Mice immunized with the GH2 vaccine exhibited similar morbidity and mortality to mice immunized with the H2 peptide alone or the unimmunized animals. Although the JH2 vaccine did not have the potency of the JH1 vaccine, these results confirmed the ability of the J-ICBL to elicit a protective T cell response.

4. L.E.A.P.S. VACCINES WITH THE GB EPITOPE FROM GLYCOPROTEIN B OF HSV-1

Glycoprotein B of HSV-1 also contains a prominent T cell epitope which is recognized by CTLs from H-2Kb expressing mice to promote killing of cells infected by either HSV-1 or HSV-2 (16). This epitope will also elicit antibody production. The 498-505 (SSIEFARL) (gB) peptide of glycoprotein B from HSV-1 was identified as a CTL target by testing a library of nested, overlapping synthetic peptides for conversion of uninfected cells into target cells (21). Cytotoxic T lymphocytes from C57BL/6 mice immunized by infection with HSV-1 recognized and killed cells which had been incubated with the 498-505 peptide.

Similar to the ICP27 vaccines, the JgB vaccine elicited protection from lethal intraperitoneal HSV challenge (Figure 3), but the GgB vaccine also appeared to elicit some protection. More than half of the JgB immunized mice survived whereas all of the control and most of the mice immunized with a control heteroconjugate (LgB) or the SSIEFARL peptide alone died within 10 days of the intraperitoneal challenge. The 'L' ICBL is a peptide from laminin/fibronectin. In addition, immunization with JgB, but not GgB or the gB peptide alone, elicited a DTH response to whole virus. This indicates that JgB promotes a Th1 type immune response and protection.

No antibody was elicited following immunization with JgB, GgB or the gB peptide, consistent with the L.E.A.P.S. vaccine being a T cell immunogen and the small size of the gB peptide. Unlike the JH1 vaccine however, antibody responses to the gB epitope were detected in mice surviving challenge with HSV. Generation of antibody to the SSIEFARL epitope required vaccination since none was detectable in unimmunized mice after challenge, despite antibodies to other viral epitopes (Table 2).

Table 2. Antibody Production In Response To Immunization With JgB And GgB And Subsequent HSV Challenge

Immunization	Challenge[1]	Anti HSV-1 gB peptide[2] O.D.	Anti HSV-1[3] O.D.
gB	+	0.043	0.035
GgB	-	0.010	0.08
GgB	+	0.065[5]	0.819[5]
JgB	-	0.013	0.093
JgB	+	0.181[5]	0.368[5]
Control[4]	+	0.038	0.490
NMS	-	0.036	0.060

[1] HSV-1 Challenge, [2] ELISA: wells coated with gB peptide (SSIEFARL), [3] ELISA: wells coated with UV inactive HSV-1, [4] Control mice received Seppic adjuvant,[5] indicates increased O.D. values (at 492nm) for post challenge, immune sera as compared to pre challenge immune sera

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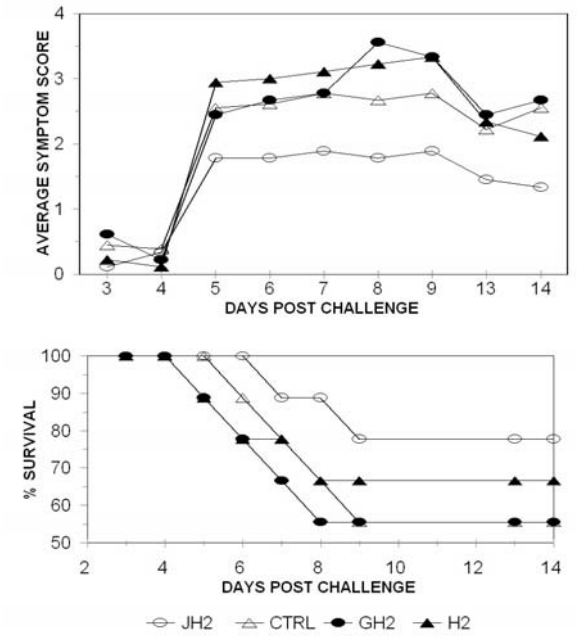

Figure 2. Immunization with JH2 protects Balb/C mice from lethal intraperitoneal HSV-1 challenge. Mice were immunized and evaluated as for Figure 1.

Although the JH1, JH2 and JgB vaccines elicited protection from lethal HSV challenge and also DTH responses, the efficacy of these vaccines is limited by the MHC restriction for recognition of the epitope incorporated into the L.E.A.P.S. heteroconjugate. The JH1 vaccine is effective in Balb/C mice whereas the JgB vaccine was effective in C57BL/6 mice. This limitation was one of the reasons for looking for another candidate epitope for inclusion in a L.E.A.P.S. anti-HSV vaccine.

5. L.E.A.P.S. VACCINES WITH THE GD EPITOPE FROM GLYCOPROTEIN D OF HSV-1

Glycoprotein D of HSV-1 is the source of a peptide that offers several advantages as the immunogen for a L.E.A.P.S. vaccine compared to the H1, H2, or gB epitopes. Glycoprotein D (gD) is required for binding and entry into the target cell and therefore is an essential glycoprotein for the virus. Antibody to this glycoprotein neutralizes the virus (blocks infection) and immunization with the glycoprotein elicits protection in mice and humans. A subunit anti-HSV vaccine currently in human trials incorporates a truncated form of gD which lacks the membrane spanning region (22). This vaccine enhanced protection against HSV-2 infection for women who were seronegative for HSV-1 but not men. It was suggested that the nature of the genital infection of a male requires a different type of immune response than for a female.

Major T cell and B cell epitopes are present in the N terminus of gD, specifically within the first thirty amino acids. We are currently developing a L.E.A.P.S. vaccine which contains a peptide consisting of amino acids 8 to 23 [gD (8-23)] of gD. This peptide elicits T cell responses as well as neutralizing antibody and the sequence is shared by the glycoprotein D of HSV-1 and HSV-2 (17). It was important to choose the correct T cell epitope from glycoprotein D for the vaccine and to initiate the correct T cell response because glycoprotein D also contains a T cell epitope which elicits suppressive responses (23) and adoptive transfer of a gD (5-23) specific Th2 T cell line exacerbated HSV keratitis in mice (24).

Immunogens incorporating either the gD (1-23) or gD (8-23) have been prepared by more conventional means. KLH conjugates of the gD peptides promoted antibody production, and elicited protection from intraperitoneal or from footpad challenge (25, 26, 27). Similarly, immunization with amino acids 1-23 in a multimeric immunogen, in which the peptide was attached to a poly L-lys-DL-Ala backbone, protected Balb/C or CBA mice against HSV-1 challenge (28). The humoral and cellular responses elicited to this peptide also protect mice from HSV-1 keratitis (29). The ability of the gD (8-23) peptide to elicit protective responses to both HSV-1 and HSV-2 in mice and humans with different MHC backgrounds was the basis for its selection for incorporation into JgD and GgD L.E.A.P.S. vaccines.

The efficacy of the JgD and GgD L.E.A.P.S. vaccines was evaluated in mice either by the intraperitoneal or the skin abrasion-zosteriform spread lethal challenge models for HSV disease (Figure 4). Unlike the JH1 and JgB vaccines, Seppic ISA-51, and not Novosomes™, was shown to be the appropriate adjuvant for the gD epitope containing vaccines. The Seppic adjuvant was also superior to the AGP 529 (a synthetic MPL S/E) adjuvant. Figure 4 shows that all of the mice immunized subcutaneously with the JgD vaccine in Seppic ISA-51 adjuvant survived challenge with an LD50 of the virulent H129 strain of HSV-1. Mice immunized with the GgD in Seppic ISA-51 in this experiment were somewhat protected

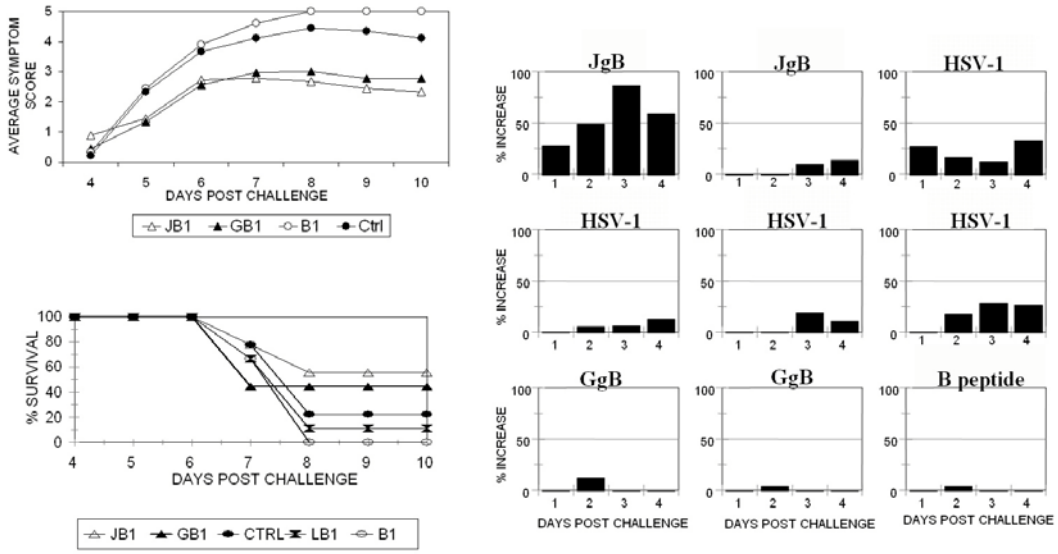

Figure 3. Immunization with JgB protects C57BL/6 mice from lethal intraperitoneal HSV-1 challenge and promotes DTH responses to virus. Mice were immunized and evaluated as for Figure 1. GgB also elicited protection.

but less so in other experiments. No protection was observed for mice immunized with the unconjugated gD (8–23) epitope in Seppic ISA-51.

The sera from surviving animals from this experiment and another independent experiment were subjected to isotype analysis for antibody specific for the gD peptide (Table 3). Serum was obtained after vaccination alone, or following vaccination and intraperitoneal challenge with HSV-1. Unvaccinated mice that survived the HSV challenge produced very weak gD (8–23) peptide specific antibodies. The formulations of L.E.A.P.S.™ vaccine in Seppic (shown by "s" in Table 3) promoted higher antibody titers in mice vaccinated with either JgD or GgD after challenge as compared to the mice receiving vaccine formulations in Novosomes (shown by "n" in Table 3). Mice immunized with JgD in Seppic ISA-51 produced no antibody until after HSV challenge and then, the IgG2a/IgG1 ratio was >1, indicative of a Th1 response. The lack of antibody production following immunization with a J- L.E.A.P.S.™ heteroconjugate vaccine without a subsequent challenge is consistent with it being a T cell immunogen, as for the JH1 (12) and the JgB peptide vaccines. The JgD immunized unchallenged mice were also capable of eliciting a DTH response to UV inactivated virus indicative of a Th1 type of immune response. Following challenge, the strength of the antibody response correlated with the severity of the disease in these mice suggesting that a greater symptom and viral load is required for the production of antibody and is actually a poor indication of immune protection.

Unlike the GH1 (ICP27) and GgB (glycoprotein B) based vaccines, GgD vaccination was sufficient to elicit a strong antibody response even without virus challenge, and the isotype was predominantly IgG1, also indicating a Th2 response. Production of IgG following immunization with GgD, without challenge, indicates that this vaccine activates both T and B cells with class switching of the type of antibody. The IgG2a/IgG1 ratio of the antibody was <1 indicative of a Th2 type of response. Development of the GgD vaccine has been deferred.

The JgD vaccine was tested further in an animal model that more closely resembles an HSV infection of humans, the skin abrasion-zosteriform spread model of HSV. After entering the skin through a mild abrasion, the virus initiates local site infection, travels to the dorsal root ganglia and then down the nerve to erupt in zoster-like blisters along the dermatome. This HSV challenge model allows the progression of infection to be visually followed and distinction of the stage of infection and disease which is blocked by the immune protection (13). Vaccination with JgD elicited 100% protection from lethal challenge and prevented disease in most of the immunized mice. Immunization of the mice with JgD protected them from local site lesions, blocked neuronal spread of the virus and prevented death (Figure 4 a,b,c). In addition, protection was elicited in Balb/C and several other strains of mice indicating that the vaccine is effective in different MHC backgrounds (Table 4).

6. IMMUNE COMPONENTS IMPORTANT FOR INITIATION AND DELIVERY OF JGD L.E.A.P.S. VACCINE PROTECTION

In an attempt to learn more about how the JgD L.E.A.P.S. vaccine initiates and delivers protection, antisera was used to ablate or neutralize specific components of the immune response elicited by JgD against HSV challenge (Table 5). These studies in Balb/C mice demonstrated that CD8 expressing cells are

Table 3. Antibody Production In Response To Immunization With JgD And GgD And Subsequent HSV Challenge.

IMMUNOGEN	IgG1	IgG2a	IgG2b	IgG3	IgG 2a/IgG1
EXP.1					
HSV-1	0	0.11[1]	0	0	>1[1]
JgD	0	0	0	0	0
JgD + challenge	0	0.94[1]	0	0.038	>1[1]
GgD	2.6[1]	0.17	0	0	0.067
GgD + challenge	3.0[1]	0.44	1.52	0.17	0.15
EXP. 2					
HSV-1	0.16	0.084	0	0	0.52
JgD(n) + challenge	0.017	0.017	0	0	too weak
JgD(s) + challenge	0.036	0.24	0	0	6.7[1]
GgD(n) + challenge	0.30	0.16	0	0	0.53
GgD(s) + challenge	2.96[1]	1.34	1.02	0.014	0.72

[1] Indicates important differences.

Table 4. JgD1 LEAPS Vaccine-Induced Protection of Different Mouse Strains

Strain[1]	Protected from zosteriform spread(%)[2]		Survival(%)[3]	
	JgD1	Control	JgD1	Control
Balb/C	71	0	71	57
C57BL/6	29	0	100	86
C3H	43	14	43	29
FVB	0	0	14	0
Swiss Webster	29	0	29	0
CD1	57	29	57	57
SKH1	43	14	57	0

[1] Each group had seven mice, [2] Block of lesion spread from primary infection, [3]Mice survivng the challenge

Table 5. Percentage Of Mice With Zosteriform Lesions After Different Treatments Before The Start of Vaccination or At The End of The Vaccination But Before Challenge

Day postinfection	Anti-CD4		Anti-CD8		Anti-IFNγ		L.E.A.P.S.		Control	
	Before vaccine	After vaccine	Before vaccine	After vaccine	Before vaccine	After vaccine	No treatment			
5	42.8[1]	71.4	57.1	28.5	57.1	85.8	28.5	28.5	71.4	57.1
6	85.8	85.8	85.8	42.8	57.1	85.8	42.8	42.8	100	71.4
7	71.4	100	85.8	42.8	71.4	85.8	42.8	42.8	100	85.8
8	85.8	100	85.8	42.8	71.4	85.8	42.8	28.5	100	100
9	85.8	100	85.8	57.1	71.4	85.8	42.8	42.8	100	100

[1] values represent percentage of mice with zosteriform lesions in each group.

important for initiating the response to JgD but CD4 expressing cells and interferon gamma are important for delivering protection. The role of CD8 cells for the initiation of the response is consistent with the ability of the J- L.E.A.P.S.™ peptide, a peptide from beta-2-microglobulin, to bind to CD8 molecules. At this point, it is not possible to say whether the relevant cells are CD8 T cells, CD8 expressing dendritic cells (30,31), or another type of cell. The importance of CD4 cells for mediating protection from HSV challenge in the immunized mice is consistent with other studies, which demonstrate their importance in protecting Balb/C mice from HSV infection (32,33). The importance of interferon gamma in mediating protection is consistent with its central role in Th1 immune responses (12) and the importance of Th1 responses in protection from HSV infection (32, 34,35).

7. SUMMARY

Development of anti-HSV vaccines utilizing the L.E.A.P.S. technology allowed the conversion of defined epitopes into immunogens which elicited protection from subsequent viral challenge. Unlike many such technologies, the nature of the subsequent immune response can be varied depending upon which ICBL/TCBL is used in the heteroconjugate vaccine. The 'J' vaccines, which elicit Th1 type immune responses, activate T cell responses but will support B cell antibody responses during viral challenge (Table 6). This is demonstrated by the presence of DTH responses and lack of antibody production following vaccination. This type of immunization is similar to the prime-boost approach that is utilized for several DNA vaccines (36). The L.E.A.P.S. vaccine appears to initiate (prime) the T cell immune response which matures (boost) upon infection or antigen presentation. The 'G' containing vaccines support Th2 type immune responses and unlike the 'J' vaccines, may be sufficient to facilitate antibody production depending upon the epitope and the peptide. These antibodies may be helpful but are not sufficient to protect against HSV infection. Ultimately, the 'G' vaccines are not useful for HSV vaccines, as indicated by their limited or lack of ability to elicit protection from infection. Future studies will be dedicated towards the development of an HSV vaccine for use in humans

Table 6. Comparison Of Response To HSV Immunogens

Immunogen	Antibody	DTH	T-cell Activity	Protection
Virus	+	+	+	+
ICP27	-	+	+	+
JH1	-	+	+	+
GH1	-	-	-	-
glycoprotein B	+	+	+	+
JgB	+[1]	+	+	+
GgB	+[1]	-	-	+
glycoprotein D	+	+	+	+
JgD	+[1]	+	+	+
GgD	+	-	-	+/-[2]

[1]Anti-peptide antibody AFTER virus challenge, [2] Variable levels of protection.

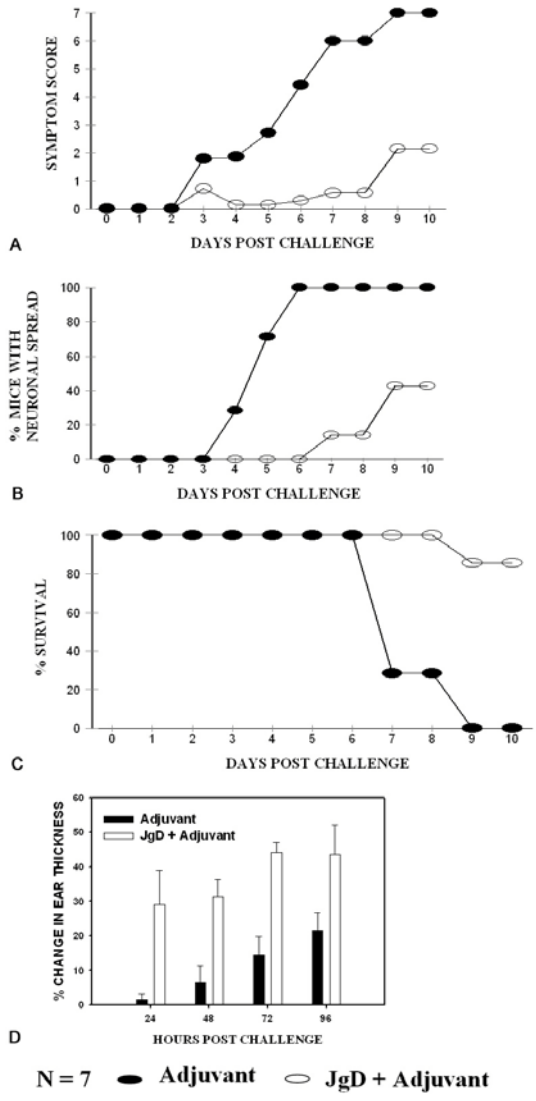

Figure 4. Immunization with JgD protects Balb/ C mice from lethal HSV-1 challenge in the skin abrasion-zosteriform challenge model. Mice were unimmunized or immunized twice with JgD in Seppic ISA-51 with a 30 day interval. After an additional 21 days, a 0.5mm patch of exposed skin was abraded with sandpaper and then infected with H129 virus. Disease progression was scored as: 0: no symptoms 1, 2: local site lesions; 3, 4: zosteriform spread of infection; 5, 6: zosteriform spread and disease progression; 7: death.

with the JgD vaccine as the lead candidate. Our studies will continue to evaluate the mechanism of action of the L.E.A.P.S. vaccines and will include the optimization of formulation, delivery methods, quality control and stability.

8. ACKNOWLEDGEMENTS

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