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THE DEVELOPMENT OF CARRIERS AND ADJUVANTS FOR USE WITH PEPTIDES TO INDUCE MUCOSAL AND SYSTEMIC IMMUNITY AGAINST BIOLOGIC TOXINS

MIDTERM REPORT

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SCIENTIFIC PROGRESS

A.RICIN: 1. Peptide vaccines:
To elicit neutralizing immunity against Ricin using analogous synthetic peptides two approaches were pursued. The first approach utilized sequences from the putative active site of the enzymatic activity (Ricin A peptides) and the second from the part of the B chain containing the major galactose binding site which facilitate cell entry (Ricin B peptides).

a. Ricin A chain:
i.Data from 16 vaccines (11 hydrophobically-linked protecsome-lipopeptide vaccines (in saline) and 5 covalently-linked KLH-peptide vaccines (emulsified in Complete Freund's Adjuvant [CFA]) representing 8 areas of Ricin A chain were analyzed and compared (see enclosed Table 1). Anti-peptide and anti-Ricin A protein IgG was successfully induced against most of the area except 199,122 and 205. The proteosome vaccine for the 161 area was significantly superior to the KLH/CFA system in inducing anti-Ricin A protein IgG and for the 190 area was superior in inducing both anti-protein and anti-peptide IgG.The titer of anti-peptide IgG induced by peptide from region 5 (199 and 205) was low and Ricin A chain protein was not recognized at all by these antibodies. None of the vaccines induced detectable neutralizing antibodies in an in vitro assay using native Ricin; nor did the peptides inhibit neutralization of Ricin A polyclonal sera.Amino acids 209 and 211 of Ricin A chain are considered critical to its enzymatic activity. Vaccines containing peptide 199-221 however were not immunogenic; although immunization with vaccines containing 190-214 did induce high-titered IgG against 190-214 (Table 1), peptide 199-221 was not well recognized by these antibodies indicating that IgG to the critical 209-211 area is still lacking. For that purpose vaccine containing peptide 205-213 was used. This vaccine was also ineffective against both the homologous peptide and Ricin A.
ii. 13 peptides from 9 areas of Ricin A chain were delivered to the Div. of Pathophysiology, USAMRIID, in order to determine if one of these areas would be recognized by a neutralizing MAb available to the USAMRIID. A peptide containing amino acids 91-117 was strongly recognized by the MAb but an overlapping peptide containing 107-132 was not indicating that the protective monoclonal interferes with Ricin A toxicity by recognizing the area contained in amino acids 91-110. On receipt of the MAb from USAMRIID, we confirmed these results.Three 10-mer peptides (91-100, 96-105 and 101-110) were synthesized, purified and tested in both direct binding and inhibition ELISAs to determine which part of 91-117 is active.None of these short peptides were active. Accordingly, a 15-mer, 96-120, and a 20-mer, 91-110, were synthesized,purified and tested as above. Both of these peptides

were positive indicating that the active site is defined by the 15-mer, 96-110.

iii. New vaccines were made using KLH or proteosome preparations that are superior in developing anti-protein antibodies (MgC-I and GC1aX-R) with peptide 91-117 that reacts with the neutralizing monoclone. Serum ELISA results are pending completion of immunization.

b. Ricin B chain:

i.Data from 8 vaccines (7 hydrophobically-linked proteosome-lipopeptide vaccines (in saline) and 1 covalently-linked KLH-peptide vaccine (emulsified in Complete Freund's Adjuvant [CFA]) representing peptides from the two homologous galactose binding site on the Ricin B chain were analyzed (see enclosed Table 2). Since two Ricin B peptides 230-257 and 244-262 from the 2nd binding site induced antibodies that recognized the homologous peptides but did not recognize native Ricin B protein, five other peptides were synthesized, purified and vaccines were made from the first putative site (20-28,20-47) and the second (232-239,247-256).These vaccines induced anti peptide but not anti Ricin B protein antibodies.

ii.Peptide 230-257 was complexed to 5 different preparations of proteosomes to determine if changed presentation of this peptide induced IgG that recognize the protein: A new lot of meningococcal proteosomes made in Israel (MgC-I), and 3 proteosome preparations made by Dr. Mylan Blake at the Rockefeller Univ., N.Y.: MgC-R, and 2 gonococcal proteosomes: GC-Std-R, and GC-X-R (lacking class 3 protein). Significantly, the standard MgC proteosome induced the highest anti-peptide titers whereas the others induced better anti-protein titers, especially MgC-I, which elicited the poorest anti-peptide titers (Table 3).Hence two new Ricin B peptide vaccines were made with the GC-X-R and MGC-I linked to 244-262 peptide which induced anti Ricin B titers of 25,000 (o.d.>0.5) with anti-peptide titers of 6,400 indicating the efficacy of this approach. Nevertheless, non of these vaccines induced detectable neutralizing antibodies in an in vitro assay using native Ricin.

2.Monoclonal Ab (MAb) production:

Neutralizing MABs to both A and B chains of Ricin can be used to identify peptides that represent neutralizing epitopes of the toxin.

a.Ricin A chain

Sera of mice immunized with Ricin A chain showed a) high titered polyclonal anti-Ricin A antibodies by ELISA and western blots and b) low-titered activity in an in vitro assay developed to test for neutralization of native Ricin. Spleens of these animals were used for fusion and 40 MAbs were identified that recognized Ricin A. Although it was initially found that many of these had neutralizing activity as well, it was subsequently shown that the this was due to a factor in the horse serum used to grow the cells. When regrown in fetal calf serum none of the 40 MABs that recognized Ricin A chain in ELISA neutralized Ricin toxicity.

b.Ricin B chain

Polyclonal antisera from mice immunized with Ricin B chain

neutralized Ricin at a titer of 1;9000 after 2 boosts. From the hundreds of Mabs that bound to Ricin B chain in an ELISA, when the two hybridomas selected for sub-cloning were expanded and grown to ascites fluid, one clone was found to be superior in neutralizing toxicity in vitro - this clone will be tested for in vivo neutralization.

B. SEB: Proteosome vaccines made from 12 lauryl-cysteine peptides were tested for induction of binding antibodies to the native SEB protein and to homologous and overlapping peptides in an ELISA. Sera were also sent to Dr. P. Gemski at WRAIR for functional assays and for comparison with KLH-covalently coupled peptide vaccines. The data are summarized in Table 4 below.

SEB is not toxic in vitro and is toxic at low doses only in man and monkey. In order to test the SEB vaccines made, new ideas for a mouse model for SEB toxicity were tested for the second time and data confirmed feasibility of the model.

C. SECRETORY IMMUNITY: Ricin A peptides RA144 and lauryl-cysteine-RA144 were purified in large quantity to make vaccines for oral immunization of pregnant rabbits to test colostrum and lung lavage for secretory IgG and IgA. The protocol included SEB alone or coupled to beads, Ricin A alone or coupled to beads, RA144 alone or coupled to beads, or lauryl-cysteine or proteosomes.Vaccines tested did not give high antibody titers in colostrum or lung lavage following oral immunization of rabbits.A murine system was developed to evaluate induction of IGA in the respiratory tract and protection against respiratory infection following intranasal immunization using peptide vaccines.Mice immunized intranasally with Ricin A or Ricin B peptides complexed to proteosomes induced low levels of specific IgA and IgG in bronchial lavage fluids and sera, respectively. Cholera toxin B chain (CTB) enhanced immunity when mixed with free Ricin A peptide or with Ricin B peptide complexed to proteosomes.A new protocol was designed to induce higher levels of antibody (2 series, 3 weeks apart, of 3 weekly immunizations). Secretory immunity experiments using shigella LPS with proteosomes to evaluate oral and intranasal immunization using antigens of known military importance indicate that at least three doses are needed for optimal responses.

TABLE 1: IMMUNOGENICITY OF RICIN A PEPTIDE VACCINES USING PROTEOSOME-LIPOPEPTIDES (in NS) or KLH-PEPTIDES (in CFA): TERTIARY MURINE IgG RESPONSES as measured by ELISA

Area	Carrier/ Adjuvant	Peptide Location (C=added Cysteine)	Reciprocal Serum Titer (O.D. > 0.5) Against	
			Ricin A Protein	Homologous Peptide
1	Proteosome	C144-170	3,200	1,600
	KLH/CFA	C144-170	6,400	1,600
2	Proteosome	161-186	3,200	3,200
	Proteosome	C161-186	3,200	3,200
	KLH/CFA	C161-186	50	6,400
3	Proteosome	C171-198	200	800
	Proteosome	C171-196	6,400	12,800
	KLH/CFA	C171-198	3,200	6,400
4	Proteosome	C190-215	51,200	6,400
	KLH/CFA	C190-215	1,600	800
5	Proteosome	C199-221	< 50	400
	KLH/CFA	C199-221	< 50	50
6	Proteosome	c91-117	1,600	3,200
7	Proteosome	C107-132	1,600	3,200
8	Proteosome	C122-148	<50	400
5a	Proteosome	C205-213	50	200

TABLE 2: IMMUNOGENICITY of PROTEOSOME-LIPOPEPTIDE (in NS) or KLH-PEPTIDE (in CFA) VACCINES from the B CHAIN of RICIN: TERTIARY MURINE IgG RESPONSES AGAINST HOMOLOGOUS RICIN PROTEIN or PEPTIDES as measured by ELISA

Ricin Area:	Carrier/ Adjuvant	Source of Peptide	Peptide Location (C=added Cysteine)	Reciprocal Serum Titer (O.D. > 0.5) Against	
				Homologous Protein	Homologous Peptide
9	Proteosome	Ricin B	C230-257	100	3,200
10	Proteosome	Ricin B	C244-262	200	1,600
10	Proteosome	Ricin B	C244-262	200	50
9a	Proteosome	Ricin B	C232-239	<50	400
10a	Proteosome	Ricin B	C247-256	50	400
11	Proteosome	Ricin B	C20-47	50	6,400
11a	Proteosome	Ricin B	C20-28	50	1,600
11b	Proteosome	Ricin B	C33-47	<50	800

TABLE 3: IMMUNOGENICITY of PROTEOSOME-LIPOPEPTIDE VACCINES from the B CHAIN of RICIN: SECONDARY and TERTIARY MURINE IgG RESPONSES AGAINST HOMOLOGOUS RICIN PROTEIN or PEPTIDES as measured by ELISA

Ricin Area:	Proteosomes: Std= standard I= Israel (new) R= Rockefeller MgC= meningo-cocci GC= gonococci X= genetically lacking Class 3:	Source of Peptide	Peptide Location (C=added Cysteine)	Reciprocal Serum Titer (O.D. > 0.5) Against			
				Homologous Protein		Homologous Peptide	
				2^0	3^0	2^0	3^0
9	Std. MgC	Ricin B	C230-257	50	50	3,200	25,600
9	MgC-I	Ricin B	C230-257	50	1,600	800	12,800
9	MgC-R	Ricin B	C230-257	<50	200	50	3,200
9	GC-Std-X	Ricin B	C230-257	100	200	800	3,200
9	GC-X-R	Ricin B	C230-257	400	400	800	1,600
10	GC-X-R	Ricin B	C244-262		25000		6,400
10	MGC-I	Ricin B	C244-262		25000		6,400

TABLE 4:

IMMUNOGENICITY OF PROTEOSOME-LIPOPEPTIDE VACCINES USING SEB PEPTIDES: TERTIARY AND QUATERNARY MURINE IgG RESPONSES AS MEASURED IN AN ELISA

No.	Peptide Location	Reciprocal Serum Titer (O.D. > 0.5) Against:		
		SEB Protein		Homologous Peptide
		tertiary	quaternary	tertiary
1	1-30	3,200	6,400	1,600
2	21-50	800	12,800	800
3	41-70	3,200	12,800	102,000
4	61-92	25,000	102,400	204,000
6	93-112	< 50	< 50	102,000
7	101-130	< 50	< 50	200
8	113-144	12,800	25,600	102,000
9	130-160	6,400	12,800	204,000
10	151-180	6,400	800	800
11	171-200	800	800	800
12	191-220	400	1,600	400
13	210-239	400	400	50

B. SECRETORY IMMUNITY: Mice were immunized intranasally with three Ricin A peptides that were recognised by the protective anti-Ricin A mAb (RA91-117, RA91-110 and RA96-110). Peptides that were hydrophobically complexed to proteosomes were found more potent in inducing anti-Ricin IgA and IgG in bronchial and intestinal lavage fluids and sera than those linked covalently to carrier protein, KLH. Priming intranasally was found superior to parenteral priming. Remarkably high anti-Ricin titers were obtained with RA96-110 complexed to proteosomes (serum titer >400 at o.d 1.0 for bronchial IgA and 200 for sera IgA in some animals).