Synthesis and Characterization of Modified Nanodiamonds for Use as a Potential Vaccine Adjuvant Delivery Platform for a Candidate Ricin Toxin Vaccine (Dataset)

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Abstract: Adjuvants are a necessary excipient in most vaccine formulations to promote efficient antigen uptake and sampling by professional antigen presenting cells (APCs) in local tissues and regional lymph nodes. Nanoparticles have the unique capacity to serve as both antigen carriers and adjuvants by virtue of their size, shape, and modifiable surface properties. Nanodiamonds represent a novel type of diamond-based nanoparticle because they have easily modified surface chemistry and high binding capacity for surface display of protein antigens. Nanodiamonds are also non-toxic but still capable of stimulating a limited inflammatory response. In this study, we modified nanodiamonds with different surface chemistries and investigated in a murine model their ability to act as an adjuvant delivery platform for a ricin subunit vaccine, RiVax. The nanodiamonds were compared to RiVax adjuvanted with aluminum salts, liposomes, and gold nanoparticles. Vaccine efficacy was assessed based on an immunity to lethal challenge model. Our studies found that although the nanodiamonds could be coated with RiVax, the resulting formulation did not improve the protective capabilities of RiVax in a murine model. However, modified nanodiamonds were also investigated and characterized and may be investigated in the future as an improvement over their unmodified counterparts.

Keywords: Nanoparticles, nanodiamonds, nanoparticle surface modification, ricin, RiVax

Key Contribution: This paper characterizes three different surface modifications of nanodiamonds (oxidized, acidified, and diamine) and showcases their changes in protein adsorption capacity for test proteins and RiVax. RiVax formulations using nanodiamonds and other nanoparticles were also tested in murine ricin challenge studies. The results from these assays can be used in the development of an improved RiVax formulation.

KU RiVax + Adjuvants
September 2019

MANTIS LAB MOUSE EXPERIMENT #510

Purpose: Adjuvant comparison using high (protective) dose of RiVax

Treatment Groups (N=6)

1- RiVax 2x10ug

2- RiVax+ alum 2x10ug (n=5)

3- RiVax+AuNP 2x10ug

4- RiVax+NaDia 2x10ug

5- Liposome RiVax 2x10ug

Control Group (n=6)

6 – Diluent & Ricin only

Experiment Day	Procedure	Date
D-40	Prime (I.P.)	7/17/2019
D-19	Boost (I.P.)	8/7/2019
D-11	Bleed (S.M.)	8/15/2019
D0	Challenge (I.P.)	8/26/2019
D1-7	Monitor Morbidity	8/27-9/2/2019

Challenge dose of 2ug/mouse is equivalent to 10x LD_{50}

All RiVax adjuvant mixtures of AuNP, NaDia, and Liposome were prepared as specified by KU

For Alhydrogel adsorption RiVax and Alhydrogel were incubated at 4C with end over end mixing for 2 hours

RESULTS:

Figure 1. Pre-challenge end-point binding curves on R'

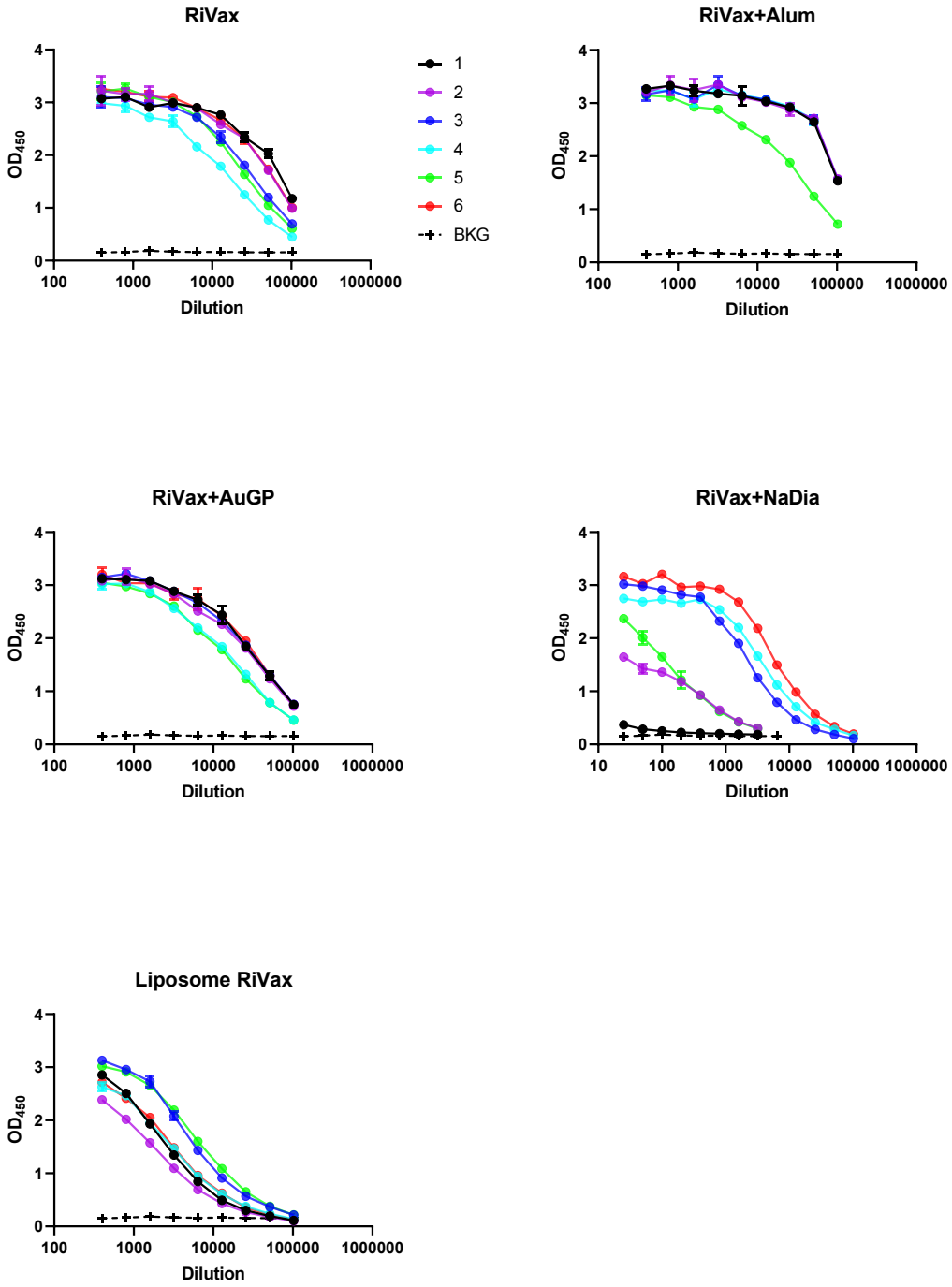

Figure 2. Serum was diluted onto plates coated with ricin holotoxin (R'). See table 2 for specific EP titer values.

Figure 2. Pre-challenge toxin neutralization assay (IC_{50} determination)

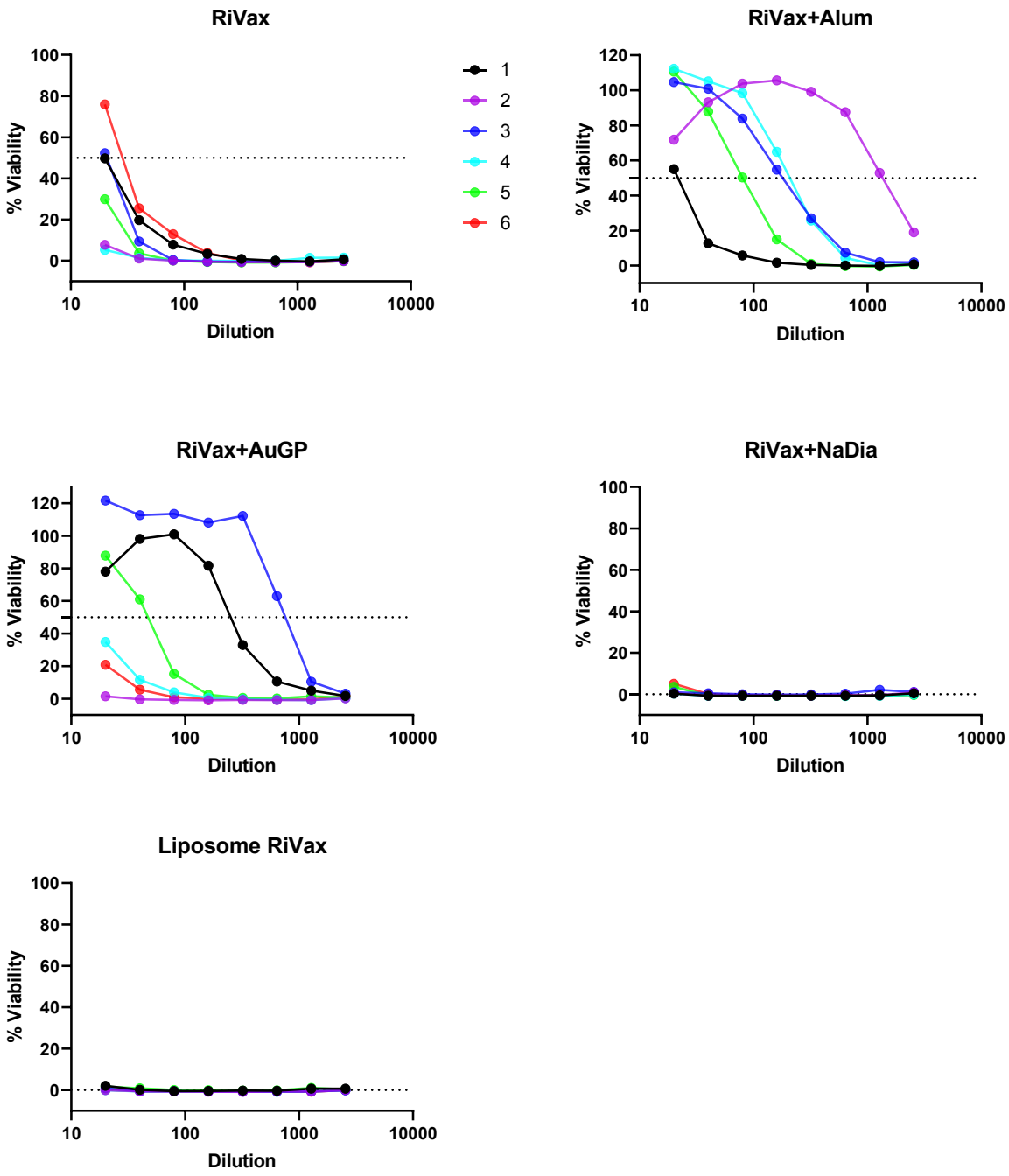

Figure 2. IC_{50} values were determined for each mouse based on toxin neutralization curves, see table 2 for values.

Figure 3. Pre-challenge end-point titers on soluble ricin (solR) vs. R' end-point titers

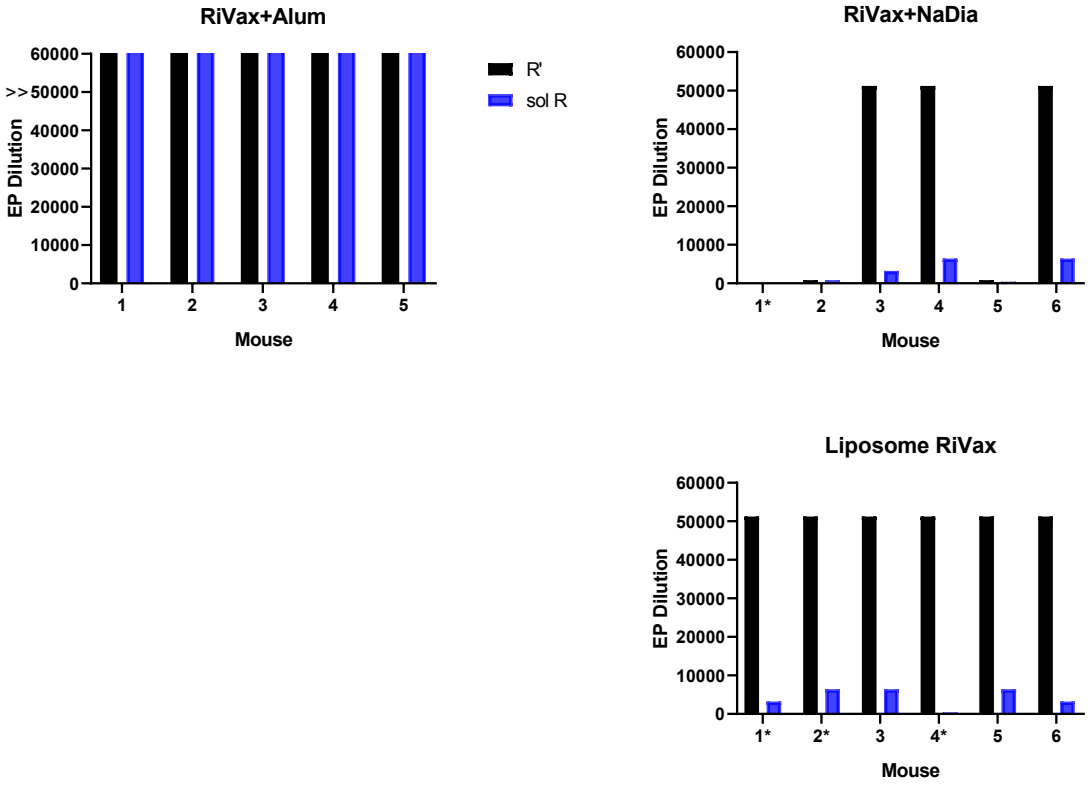

Figure 3. Despite high serum EP titers on R', RiVax+NaDia and liposome RiVax mice displayed no toxin neutralizing capabilities, this suggested that the anti-ricin antibodies present in these mice did not recognize soluble (native) ricin. In order to determine if the ricin-specific polyclonal antibodies raised in these mice bind soluble holotoxin, EP titers were determined on captured (soluble) ricin. As suspected, there are stark difference in pAb binding to solR (blue) versus non-native R'(black) for both NaDia and liposome adjuvants. RiVax+Alum serum was used as R' & solR binding assay control. See table 2 for specific solR EP titers.

Figure 4. Post-challenge survival

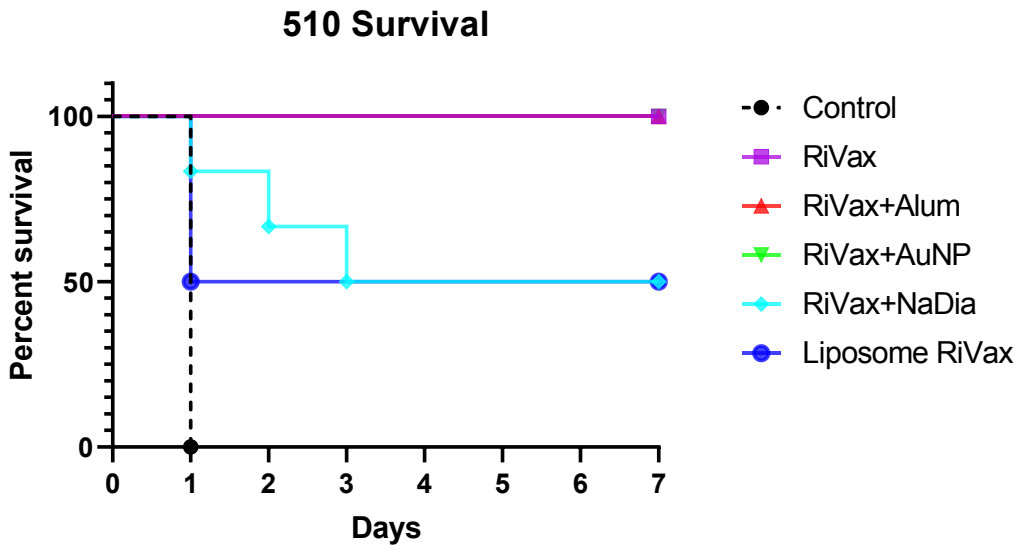

Figure 1. Kaplan-Meier survival curve. Survival differences are significant: RiVax, RiVax+Alum, RiVax+AuNP (100%); versus RiVax+NaDia & liposome RiVax (50%); p=0.0001 log-rank Mantel-Cox test.

Table 1. Post-Challenge mouse morbidity.

Day		G1	G2	G3	G4	G5	G6
1	Ruffle	+	-	+	++	+	+++
	Hunch	+	-	+	++	+	+++
	weakness	-	-	-	+	+	+++
	tremor	-	-	-	+	-	++
2	Ruffle	-	-	-	++	-	
	Hunch	-	-	-	++	-	
	weakness	-	-	-	+	-	
3	Ruffle	-	-	-	++	-	
	Hunch	-	-	-	+	-	
4	Ruffle	-	-	-	+	-	

No signs of morbidity days 5-7 for any surviving mice. Control mice (G6) succumbed to intoxication by 30 hours post challenge.

Table 2. Experimental data summary

Mouse	Treatment	EP titer (R')	EP titer (solR)	TNA (IC_{50})	Survived
1.1	RiVax	>102400	NT	20	Y
2.1	RiVax	>102400	NT	<LOD	Y
3.1	RiVax	>102400	NT	20	Y
4.1	RiVax	>102400	NT	<LOD	Y
5.1	RiVax	>102400	NT	10	Y
6.1	RiVax	>102400	NT	20	Y
1.2	RiVax+Alum	>102400	>>51200	20	Y
2.2	RiVax+Alum	>102400	>>51200	1280	Y
3.2	RiVax+Alum	>102400	>>51200	160	Y
4.2	RiVax+Alum	>102400	>>51200	160	Y
5.2	RiVax+Alum	>102400	>>51200	80	Y
1.3	RiVax+AuGP	>102400	NT	160	Y
2.3	RiVax+AuGP	>102400	NT	<LOD	Y
3.3	RiVax+AuGP	>102400	NT	640	Y
4.3	RiVax+AuGP	>102400	NT	10	Y
5.3	RiVax+AuGP	>102400	NT	40	Y
6.3	RiVax+AuGP	>102400	NT	<LOD	Y
1.4	RiVax+NaDia	<LOD	<LOD	<LOD	N
2.4	RiVax+NaDia	800	800	<LOD	N
3.4	RiVax+NaDia	51200	3200	<LOD	Y
4.4	RiVax+NaDia	51200	6400	<LOD	Y
5.4	RiVax+NaDia	800	400	<LOD	N
6.4	RiVax+NaDia	51200	6400	<LOD	Y
1.5	Liposome RiVax	51200	3200	<LOD	N
2.5	Liposome RiVax	51200	6400	<LOD	N
3.5	Liposome RiVax	51200	6400	<LOD	Y
4.5	Liposome RiVax	51200	400	<LOD	N
5.5	Liposome RiVax	51200	6400	<LOD	Y
6.5	Liposome RiVax	51200	3200	<LOD	Y

Red indicates non-survivors