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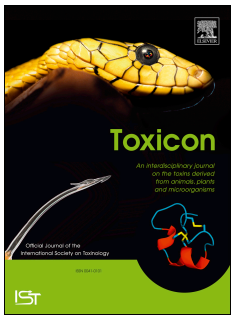

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Letter to the editor

Neutralization capacity of recombinant antivenoms based on monoclonal antibodies and nanobodies

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To the editor

Snakebite envenoming has received renewed attention in recent years, and several research programs have been initiated with the aim of improving envenoming therapy[1,2]. Traditionally, antivenoms are derived through hyper immunization of large animals, *e.g.* horses, with whole venoms, followed by purification of the generated antibodies. Although these antivenoms continue to save multiple lives, they come with several drawbacks, including a high cost of production, batch-to-batch variation, dependence on venoms, a low amount of therapeutically active antibodies, and the risk of causing severe adverse reactions when given to patients. Several projects have been initiated to improve these existing plasma-derived antivenoms using strategies such as a more effective immunization[3–5]. As an alternative, other efforts focus on the development of recombinant antivenoms based on monoclonal antibodies and fragments thereof[6–10]. One strategy for the discovery of monoclonal recombinant antibodies involves the use of *in vitro* display technologies[11], such as antibody phage display[12]. This technology enables the discovery of monoclonal antibodies from large antibody repertoires without the need for human or animal immunization. In addition, phage display technology can be employed with antibody repertoires from immune sources, which has resulted in discovery of neutralizing nanobodies and human monoclonal antibodies[13,14].

Within the snake toxinology community, a question has been raised in scientific debates of whether it is possible to generate recombinant antivenoms with similar neutralization capacities as plasma-derived polyclonal antivenoms. In the light of such discussions, we wish to point out that recent reports demonstrate that it is indeed possible to discover broadly-neutralizing monoclonal antibodies and nanobodies with sub-nanomolar affinities towards snake toxins from multiple snake species[7,10,13,14]. It has further been shown that higher affinity of these antibodies and nanobodies correlates with improved neutralization capacity both *in vitro* and *in vivo*[7,10,14]. This means that recombinant monoclonal antibodies,

when developed correctly, are able to neutralize toxins at molar ratios of 1:1 (antibody:toxin) or even lower[10,14].

Currently, no sophisticated models exist that can account for the complex toxicokinetics and pharmacokinetics at play when a snakebite is treated with antivenom. Therefore, the neutralization capacity of traditional antivenoms is typically evaluated by assessing how many LD_{50}s or mg of venom an antivenom can neutralize in a rodent, when antivenom and venom are preincubated and administered intravenously[15]. Using the same principles, the neutralization capacity of recombinant antivenoms can also be estimated. Recently published data has shown that an antibody administered in a 1:1 (antibody:toxin) molar ratio or lower can effectively neutralize whole venom *in vivo*, providing a neutralizing capacity of at least 97 mg of venom per g of IgG antibody[10] or at least 241 mg of venom per g of dimeric nanobody-Fc (Table 1)[14]. In this relation, it is to be noted that for neutralization of venoms with multiple medically relevant toxins, an oligoclonal mixture of recombinant antibodies is needed[6,16]. While this does not force the neutralization capacities of the antibodies to change[17], this aspect does set requirements for how the specific composition of the antibody mixture is defined[18]. Nonetheless, these calculated neutralization capacities compare favorably with reported *in vivo* neutralization capacities of conventional antivenoms (Figure 1), which for elapids are reported to possess neutralizing capacities, estimated as ED_{50}s, between 2–26 mg of venom per g of IgG-based antivenom (Table 2)[19]. For these calculations, it must, however, be highlighted that different challenge doses of venoms were used for recombinant antibodies (2 LD_{50}s) and plasma-derived antivenoms (2 or 3 LD_{50}s). Nevertheless, we conclude that lower amounts (w/w) of recombinant antivenoms based on monoclonal antibodies and/or nanobodies can achieve a similar or better level of *in vivo* neutralization of snake venoms than traditional antivenoms derived from the plasma of immunized animals using preincubation

models. We further hypothesize that these preclinical findings may translate into higher efficacy in the clinical setting, although this remains to be tested.

Conflicts of Interest: The authors declare no conflict of interest.

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Table 1. Neutralizing capacity of recombinant antivenoms

Recombinant Antibody	Antibody format	Venom	Amount venom (µg/mouse)	Amount antibody (µg)	Neutralization (mg_{venom}/g_{AV})
2554_01_D11[10]	IgG	*N. kaouthia*	9	94	97
V_HH2-Fc C2[14]	V_HH2-Fc	*N. kaouthia*	12[a]	31	241[b]

[a] 4 µg/mouse of α-cobratoxin is assumed to represent the 53% of long α-neurotoxins present in whole venom of *N. kaouthia*[20].

[b] V_HH2-Fc has a molecular weight of about 80 kDa compared to 150 kDa for IgG. Therefore, it has a higher neutralization capacity on a w/w basis.

147 **Table 2. Neutralizing capacity of traditional serum derived antivenoms**

Venom	Type	Venom origin	Antivenom (AV)	AV Format	Average AV conc. (mg/mL)	Venom dose	ED_{50} (mg_{venom}/mL_{AV})	Neutralization (mg_{venom}/g_{AV})[a]	Neutralization (mg_{venom}/g_{AV})[b]	Reference
N. nubiae	CYT elapid	N.S.	Antivipmyn-Africa	F(ab)'2	28.0	3 LD_{50}	0.22	7.9	5.2	21
D. viridis	NT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.35	12.5	8.3	21
N. nivea	NT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.47	16.8	11.2	21
N. haje	NT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.55	19.6	13.1	21
N. melanoleuca	NT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.56	20.0	13.3	21
N. pallida	CYT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.57	20.4	13.6	21
D. polylepis	NT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.59	21.1	14.0	21
N. annulifera	NT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.59	21.1	14.0	21
N. nigricollis	CYT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.81	28.9	19.3	21
N. mossambica	CYT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.84	30.0	20.0	21
N. katiensis	CYT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.86	30.7	20.5	21
H. haemachatus	CYT elapid	N.S.	Antivipmyn-Africa	$F(ab)'_2$	28.0	3 LD_{50}	0.91	32.5	21.7	21
D. polylepis	NT elapid	Eswatini	ASNA antivenom C	$F(ab)'_2$	44.0	3 LD_{50}	0.20	4.5	3.0	22
N. pallida	CYT elapid	Kenya	EchiTAb-Plus-ICP	IgG	56.4	3 LD_{50}	0.38	6.7	6.7	23
N. nigricollis	CYT elapid	Nigeria	EchiTAb-Plus-ICP	IgG	56.4	3 LD_{50}	0.50	8.9	8.9	23
N. mossambica	CYT elapid	Tanzania	EchiTAb-Plus-ICP	IgG	56.4	3 LD_{50}	0.66	11.7	11.7	23
N. nigricollis	CYT elapid	Cameroon	EchiTAb-Plus-ICP	IgG	56.4	3 LD_{50}	0.70	12.4	12.4	24
N. nigricollis	CYT elapid	Nigeria	EchiTAb-Plus-ICP	IgG	56.4	3 LD_{50}	1.28	22.7	22.7	24
N. melanoleuca	NT elapid	N.S.	Fav-Afrique	$F(ab)'_2$	101.2	3 LD_{50}	0.36	3.6	2.4	25
D. polylepis	NT elapid	Eswatini	Fav-Afrique	$F(ab)'_2$	101.2	3 LD_{50}	0.70	6.9	4.6	22
N. annulifera	NT elapid	Eswatini	Fav-Afrique	$F(ab)'_2$	101.2	3 LD_{50}	0.90	8.9	5.9	22
N. mossambica	CYT elapid	Eswatini	Fav-Afrique	$F(ab)'_2$	101.2	3 LD_{50}	1.40	13.8	9.2	22
H. haemachatus	CYT elapid	Eswatini	Fav-Afrique	$F(ab)'_2$	101.2	3 LD_{50}	1.50	14.8	9.9	22

A.s.intermedius	NT elapid	N.S.	SAIMR Polyvalent	F(ab)'$_2$	147.8	2 LD_{50}	0.47	3.2	2.1	26
N. mossambica	CYT elapid	Eswatini	SAIMR Polyvalent	F(ab)'$_2$	147.8	3 LD_{50}	1.40	9.5	6.3	22
N. annulifera	NT elapid	Eswatini	SAIMR Polyvalent	F(ab)'$_2$	147.8	3 LD_{50}	1.80	12.2	8.1	22
N. nivea	NT elapid	N.S.	SAIMR Polyvalent	F(ab)'$_2$	147.8	2 LD_{50}	1.89	12.8	8.5	26
D. polylepis	NT elapid	Eswatini	SAIMR Polyvalent	F(ab)'$_2$	147.8	3 LD_{50}	2.30	15.6	10.4	22
D. polylepis	NT elapid	Kenya	SAIMR Polyvalent	F(ab)'$_2$	147.8	3 LD_{50}	5.26	35.6	23.7	22
H. haemachatus	CYT elapid	Eswatini	SAIMR Polyvalent	F(ab)'$_2$	147.8	3 LD_{50}	5.70	38.6	25.7	22

[a] Neutralization is calculated as: $\frac{ED_{50}\ (\frac{mg\ venom}{mL\ AV})}{Average\ AV\ conc.(\frac{mg}{mL})}$. Average venom concentration and ED_{50} values are obtained from Ainsworth *et al*[19].

[b] Antivenoms consisting of F(ab)'$_2$ are normalized to the theoretical neutralizing capacity as IgG using a molecular weight of 150 kDa and 100 kDa for IgG and F(ab)'$_2$, respectively.

157 **Figure 1.** Neutralizing capacity for plasma-derived antivenoms and recombinant antivenoms. Neutralization is presented as mg of venom
158 neutralized per g of IgG or V_HH2-Fc.

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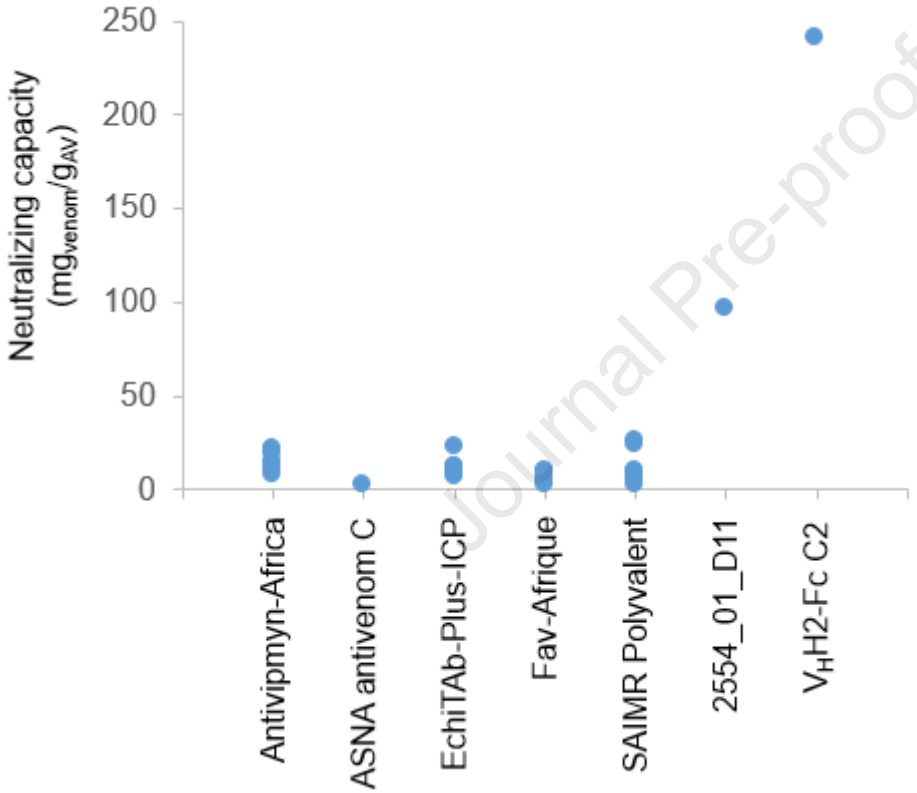

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Highlights

- Neutralization capacities are provided for recombinant snakebite antivenoms
- Neutralization capacities are compared between conventional and recombinant antivenoms
- Recombinant antivenoms may have higher neutralization capacities than conventional antivenoms

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: