Supplement file

Long-term memory response after a single intramuscular rabies booster vaccination, 10-24 years after primary immunization

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Supplement content:

Supplement file 1

Study participants

Healthy volunteers of 18 years and older were recruited if they met the inclusion criteria. All participants had received rabies PrEP at least ten years before enrolment and had never received a rabies booster immunization, or rabies immunoglobulins. Exclusion criteria were immune-deficiencies, allergies to components, pregnancy, breastfeeding, and using mefloquine or chloroquine as malaria prophylaxis. Note: Since 2020, malaria prophylaxis is no longer considered contra-indicated. After an automated search in the military medical records, potential military participants were approached for the study. Other civilian participants were identified from the travelers database of the Center for Tropical Medicine and Travel Medicine.

Supplement file 2

Explanation of exclusion

We excluded one 50 years old male subject. According to the medical files, he had supposedly received an administration of three IM rabies immunization 20 years before, but evidence of this vaccination was lacking, as he had lost his individual vaccination passport. He was not immunocompromised, nor had he been using immunosuppressive drugs. His titers on day 0, 3, 7 and 14, were 0.17, 0.17, 0.17 and 0.61 IU/ml, respectively. On day 28, we collected an additional sample and performed the RFFIT again, resulting in a titer of 0.61 IU/ml.

This immunogenicity data over these five time-points were suggestive of a primary response, rather than a secondary or booster response. Therefore, we concluded that the IM booster immunization probably was his first rabies immunization ever; so he was excluded from the study. To prove this hypothesis, it would have been ideal to check for a secondary response with a new booster immunization after 1 year, however, this is beyond the scope of the research reported here.

Supplement Table S1: base line characteristics for total and per group

Group	Subjects (n)	Age in years median [range]	Sex (n)	Interval in years median [range]	Availability of immunogenicity data (n) Day	 % ≥0.5 IU/ml	 GMT, 95% CI, range[a]
TOTAL	28	42.5 [30-63]	Male: 18 Female: 10	11 [10-24]	0 3 7 14	28 28 28 28	24 23 28 28
3-IM Group	9	50 [43-57]	Male: 9 Female: 0	23 [14-24]	0 3 7 14	9 9 9 9	9 9 9 9
3-ID Group	10	35 [30-63]	Male: 3 Female: 7	10 [10-12]	0 3 7 14	10 10 10 10	9 9 10 10
DIV Group	9	39 [33-52]	Male: 6 Female: 3	10 [10-12]	0 3 7 14	9 9 9 9	6 5 9 9

[a] GMT, 95% CI and range in subjects with adequate (≥0.5 IU/ml) titer

Abbreviations: CI: confidence interval; DIV: divergent; GMT: geometric mean titer; ID: intradermal; IM: intramuscular

Supplement Table S2: Characteristics in divergent group per participant on baseline

Divergent PrEP-schedule	Days of immunization	ROA	Interval (in years)	Sex	Age (in years)
3 dose					
	0 – 7 – 36	ID (0.1 ml)	10	M	52
	0 – 11 – 21	ID (0.1 ml)	12	M	36
	0 – 12 – 28	ID (0.1 ml)	11	M	33
2 dose					
	0 – 7	ID (0.1 ml)	11	M	34
	0 – 7	IM (full dose)	10	M	34
	0 – 7	IM (full dose)	11	F	42
	0 – 8	IM (full dose)	10	F	46
	0 – 11	IM (full dose)	10	M	39
	0 – 11	IM (full dose)	10	F	41

Abbreviations: ID: intradermal; IM intramuscular; PrEP: pre-exposure prophylaxis; ROA: route of administration

Supplement Table S3: Rabies antibody response after boostering a 2-dose PrEP-schedule 10-11 years before; immunogenicity data per time-point

	Time-point	≥0.5 IU/ml	GMT (95% CI)[a]	Range[a]	≥ 3 IU/ml	≥ 10 IU/ml	Fold increase
2-dose	day 0	83%	2.33 (0.31 - 3.86)	0.50 - 5.87	17%	0%	1.00
	day 3	67%	1.81 (0.60 - 5.52)	1.07 - 5.08	17%	0%	0.78
	day 7	100%	30.68 (13.59 - 69.25)	12.89 - 110.87	100%	100%	13.17
	day 14	100%	122.32 (42.79 - 349.62)	39.62 - 391.57	100%	100%	52.50

[a] in subjects with adequate titers (≥0.5 IU/ml) in international units per milliliter

Abbreviations: CI: confidence interval; GMT: geometric mean titer; PrEP: pre-exposure prophylaxis