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Surrogate markers of immunity to *Leishmania major* in leishmanin skin test negative individuals from an endemic area re-visited

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Abstract

Background: In the screening of vaccine candidates it is important to select candidates that evoke immune responses associated with protection. Valid surrogate markers against human leishmaniasis are still lacking.
Methods: A controlled injection of live *Leishmania* known as leishmanization, (LZ), was used to evaluate vaccine (alum-precipitated autoclaved *Leishmania major* with BCG) efficacy and more accurately define surrogate markers of immunity to leishmaniasis in humans.

Cellular immune responses to this artificial infection were monitored in the volunteers prior to and 9 months post infection. Comparisons were made between those who developed a lesion after infection and those who did not not.
Results: In the volunteers monitored there was no significant difference in LST, IFNγ production, or source of IFNγ between those who developed a lesion and those who did not after LZ, with the exception that ulcer development was associated with an enhanced number of IFNγ secreting $CD4^+$ $CD45RA^-$ (memory) T cells.
Discussion: Ulcer development following LZ was lower than anticipated by a pilot study (47% versus 78%) using the same stabilate several years earlier. While this may be an effect of low viability/virulence of the LZ inocula, alternative explanations are also possible. The IFNγ responses in the study subjects were significantly lower compared to volunteers with previous history of cutaneous leishmaniasis. The findings raise the possibility that the selection of LST-negative volunteers in an endemic area may bias the study towards potentially non/low *L. major*-reactive volunteers.

Keywords: *Leishmania*; LZ; vaccine; LST; IFNγ; T cell

1. Introduction

Leishmaniasis are a group of parasitic diseases caused by *Leishmania* species with manifestations that range from a self-healing cutaneous lesion to mucocutaneous, to a lethal systemic visceral form of the disease. Spontaneous or drug-cured patients seldom show a recurrence or re-infection of diseases. In addition, as it is possible to protect various animals by vaccination [1], a vaccine for humans would appear to be achievable. Historically protection against cutaneous leishmaniasis (CL) has been achieved through deliberate infection using live parasites at a preferred body site — a procedure known as LZ [2]. This method utilising *Leishmania major* promastigotes has been practised on large number of people in the South-Central, South Western part of Asia and Israel [3,4]. Although, highly protective, this form of vac-

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cination has been debated partially due to loss of virulence [4] and development of active lesion, which sometimes last for long time requiring treatment. LZ is to date only routinely practised in Uzbekistan with freshly isolated parasites annually. Currently there is no vaccine available against any form of leishmaniasis. Killed promastigotes have been tested in a number of clinical trials with variable results [5–8]. It has been suggested that the performance of the killed promastigotes may be improved by using an appropriate adjuvant. Common for all these vaccine studies is that the volunteers have been selected based on no LST, since an LST reaction is believed to be a sign of previous exposure to *Leishmania* antigens and classically assumed to be a marker of protection. Another consideration has been safety. Injection of antigens to which an individual exhibits a delayed type hypersensitivity response, represents a risk that is better avoided before the recipient is known to be at risk of infection and the vaccine is shown to be protective. Volunteers with no response to LST have accordingly been considered as non-immune or not exposed, thus, susceptible to infection and appropriate candidates for evaluation of vaccines.

Protection against leishmaniasis in *L. major* mouse models has been associated with Th1 type response, i.e. CD4 T cell-dependent IFNγ secretion, whereas susceptibility has been associated with production of Th2 type response, reviewed by Sacks and Noben-Trauth [1]. Studies using mononuclear cells from humans recovered from leishmaniasis have associated similar mechanisms with cure of leishmaniasis [9–11]. The typical Th2 pattern has been less obvious in humans, however, non-healing and severe forms of leishmaniasis have been associated with IL-10 production [11–13]. The general consensus is that an ideal vaccine against leishmaniasis in humans would be expected to induce an appropriate Th1 type response in the absence of a strong Th2 type response and thereby protect against challenge (natural or artificial).

Although, several studies have indicated that development of LST, production of IFNγ/IL-10, or lack of such could serve as markers of immunity to human leishmaniasis, true predictive markers of protection against leishmaniasis remain elusive. This study was set to identify responses that could be predictive for the outcome of the disease. The unique situation of knowing the exact time of infection made it possible to follow the dynamics of the cellular responses that have previously been associated with protection and disease progression in human leishmaniasis. Such markers would be most useful when evaluating vaccine candidate molecules. However, due to highly reduced and over time wide spread LZ take rate compared to previous trials and the limited availability of samples for immunological studies, our data should be regarded as preliminary.

To this end cytokine secretion and the identification of the cellular source of cytokines have been evaluated before and after infection in a limited number of samples.

The data may be interpreted by the suggestion that these LST-negative volunteers from endemic foci lack or have reduced capacity to mount leishmanial antigen-specific T cell IFNγ responses. We suggest that the decision to select LST-negative volunteers from leishmaniasis endemic foci for vaccine efficacy studies should be re-visited. This is particularly important since naturally LST converted individuals without a history of leishmaniasis in an endemic area are not fully protected against the disease (Khamesipour et al., in preparation).

Table 1
Study group vaccination received, LST reaction before leishmanization

Study number	Sex	Age	Vaccination (mg ALM)	LST (mm)
810	F	34	10	0
821	M	19	10	nd
840	M	17	10	4.5
842	F	16	320	5
869	F	19	320	0
894	M	20	200	5
895	M	29	Diluent	nd
907	F	21	100	5,5
908	F	17	320	3,5
912	F	19	10	2,5
920	F	20	Diluent	0
922	M	17	10	0
003	F	32	–	0
002	M	35	–	0
005	F	37	–	0
006	M	22	–	0
806	F	27	Diluent	0
873	F	39	Diluent	0
902	M	51	320	5
831	M	54	Diluent	0
848	F	30	320	nd
854	F	28	320	0
860	F	16	Diluent	0
863	F	42	320	2
865	M	33	Diluent	0
870	M	26	Diluent	0
879	F	40	10	0
898	M	24	100	0
899	F	48	320	5
911	F	20	Diluent	0
913	F	32	100	0
931	M	25	320	0
932	F	39	Diluent	0

F: female, M: male.

2. Materials and methods

2.1. Study design and selection of volunteers

The present study was a part of a randomised double blind field trial where efficacy of the alum-precipitated autoclaved *L. major* (alum-ALM) mixed with a 10th of normal dose of BCG was evaluated against live challenge with *L. major*. The study evaluating the vaccine will be reported separately (Khamesipour et al., in preparation), although LST conversion data and IFNγ responses pre-LZ, post vaccine, for donors included in this study are shown in Tables 1 and 2, respectively.