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Brief Communications

A COMPARISON BETWEEN THE PROTEIN PROFILE OF INTACT AND TWO PREPARATIONS OF KILLED *Leishmania major* BY SODIUM DODECYL SULFATE POLYACRYLAMIDE GEL ELECTROPHORESIS

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Leishmaniasis is caused by species of the genus *Leishmania*. According to the species involved and host immune responses, human disease varies from a simple self-healing cutaneous lesion to complicated visceral or mucocutaneous forms.[1] The total world population at risk is estimated to be about 350 million. According to the WHO/TDR, approximately 1.5 million new cases of leishmaniasis are reported annually and the number of people affected is growing.[2] In India during 1977-1978, there was an estimated 20,000 deaths as a result of visceral leishmaniasis.[3] Due to large epidemics in India and Sudan, it is estimated that visceral leishmaniasis may have killed 75,000 people in 1991.[4]

Researchers have long been in pursuit of methods which may control the disease.[5,6] It is generally believed that the sole measure to control leishmaniasis is to find an effective vaccine.[7]

Initially in Iran with WHO/TDR support, two preparations of *L. major*, killed either by thimerosal (KLM) or autoclave (ALM), were compared for safety and immunogenicity. Based on preliminary results and because ALM is easier to use under field conditions, it was decided to use ALM as a vaccine in field efficacy trials. In order to reveal any possible differences in the protein profile of KLM, ALM and intact *L. major*, they were compared by one-dimensional SDS-PAGE under reducing conditions.[8,9]

Both preparations (ALM and KLM) were produced at Razi Institute of Iran as previously reported.[10] Briefly *L. major* (MRHO/IR/75/ER) was cultivated in RPMI with 15% FCS at 25°C. Promastigotes were harvested by centrifugation at stationary phase and washed five times with PBS. The pellet was resuspended and adjusted to the desired protein concentration. The preparation was divided into two parts. One part (ALM) was autoclaved at 121°C for 30 minutes and the other part (KLM) was treated with 1:10,000 thimerosal and freeze-thawed several times.

SDS-PAGE was used to determine the protein profile of ALM, KLM and intact *L. major*. This method is a powerful tool for separation and characterization of proteins and has been widely used. The samples were boiled for 5 minutes in sample buffer in the presence of SDS and 2-mercaptoethanol, and then electrophoresed under the following conditions:

1. Gel concentration, 13%
2. Current voltage, 60 until penetration of the samples into the stacking gel, and 120 thereafter
3. Time of electrophoresis, 5 hours
4. Inoculum. 20 μL
5. Protein concentration, 50 μg/20 μL
6. Finally the slab gel was stained by 0.1% Coomassie blue R-250.

As shown in Fig. 1, KLM lanes 2 and 5, in gross is the

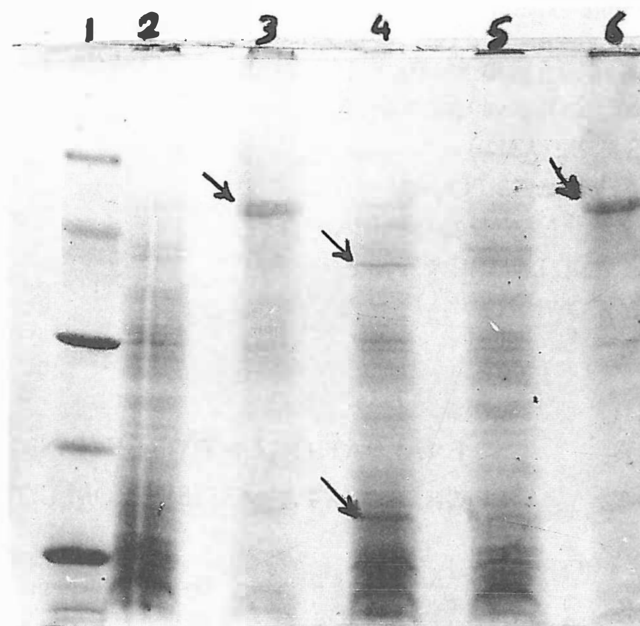

Fig. 1. Comparison of vaccine protein profiles (KLM & ALM) and native *L. major* by one-dimensional SDS-PAGE.
Lane 1: Molecular weight markers contain phosphorylase B= 94,000 Da, bovine serum albumin= 67,000 Da, ovalbumin= 43,000 Da, carbonic anhydrase= 30,000 Da, soybean trypsin inhibitor= 20,000 Da, and α-lactalbumin= 14,400 Da.
Lane 3,6: ALM; Lane 2,5: KLM; Lane 4: native *L. major*.

mirror image of *L. major* (lane 4), except for two bands at 57 kDa and 24 kDa (see arrows, lane 4) that are more prominent in intact *L. major* than in KLM; otherwise the remaining bands are more or less similar.

In ALM, lanes 3 and 6, there are two bands at 71 kDa (see arrows, lanes 3 & 6) close to each other that are absent in intact *L. major* and KLM. At the same time there are areas from 71 kDa to 43 kDa and from 43 kDa to 14 kDa that contain very faint bands as compared to intact *L. major* and KLM. The prominent bands in ALM may be aggregations of proteins due to heat, or further breakdown to smaller molecules which were eluted from the gel.

In an experiment in a murine model, ALM showed promising results in comparison with KLM and intact *L. major*, and since ALM vaccine is under an efficacy field trial locally in Iran (Isfahan and Bam) and abroad in Pakistan and Sudan, it was essential to characterize the antigenic profile of different preparations.

According to the results of this experiment, it seems that the autoclave process induces some alteration in ALM protein profile when compared with KLM or intact *L. major*. This alteration induced more protection in the murine model (data in press) and so ALM might be more suitable for use in efficacy field trials in endemic areas. In order to determine the molecules which are responsible for protection in the murine model, further experiments are needed to use ALM bands which are absent in KLM or intact *L. major*. In the future experiments, various doses, routes and adjuvants must be tried. In parallel experiments, KLM bands which are absent in ALM might be used to determine any possible role for immunosuppression.

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