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(Avid) rosette-forming cells as evidence for cellular immunity following measles virus vaccination in monkeys: correlation with the presence of haemagglutination inhibitory antibodies

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Summary

Lymphocytes characterized by in vitro rosette formation with sheep erythrocytes were enumerated in blood of normal and measles virus-vaccinated monkeys. Rosetting lymphocyte counts increased significantly as a result of vaccination. Concomitantly, HAI-antibody, undetectable before vaccination, became detectable, suggesting that the simple and inexpensive technique of enumerating rosetting lymphocytes can be used as an alternative to other more cumbersome and expensive methods for evaluation of development of cell-mediated immunity following measles virus vaccination.

Measles virus; Cell-mediated immunity

Introduction

Host defence against viral infection appears to be mediated by various types of humoral or cellular effector mechanisms, usually acting in cooperation and forming a complex network (Bloom and Rager-Zisman, 1975). Cell-mediated immunity (CMI) is considered the most effective mechanism of recovery from many viral infections (Yamanaka et al., 1976).

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A number of in vitro and in vivo methods had been employed to study the cellular immune reactions to a variety of viruses. For example, Mizutani et al. (1971) and Gerson and Haslam (1971) studied delayed type hypersensitivity skin reactions elicited by viral antigens. Macrophage migration (Mizutani et al., 1971) and blastoid transformation of lymphocytes (Saunders et al., 1969; Klajman et al., 1973) have also been used. More recently, a number of workers (Labowskie et al., 1974; Steel et al., 1973; Chiba et al., 1974; Yamanaka et al., 1976) have developed in vitro lymphocytotoxicity assay systems employing ^{51}Cr-release to assess CMI against measles and rubella viruses. These various assays, though very reliable, are quite difficult to perform, especially in developing countries, where specialized equipment is often not available.

We have used enumeration of rosette-forming lymphocytes to evaluate the cellular immune response to measles virus. Here, we show that this technique, which is inexpensive and easy to perform, represents a potential alternative to more cumbersome and expensive methods.

Experimental

The experiment was done with 5 young patas monkeys (*Erythocebas patas*) obtained locally. These monkeys were initially seronegative by haemagglutination inhibition (HAI) test to measles virus. The analysis covers results obtained before and after vaccination with Schwarz measles virus vaccine (Batch No. LM22A01, Pasteur Institute, obtained from the Epidemiological Unit of the Kaduna State Ministry of Health, Zaria). The animals received a single dose of 0.5 ml containing 100 $TCID_{50}$ according to the manufacturer's recommendation. Boosters were given twice at weekly intervals. Blood samples were collected prior to and 3 weeks after immunization.

Approximately 5 ml of venous blood was collected from each monkey into tubes containing 250 units of heparin. The samples were immediately diluted with an equal volume of PBS, and the mononuclear cell layer was obtained by centrifugation (1000 × *g*, 30 min, room temperature) on Ficoll-Hypaque gradients (Pharmacia, Uppsala, Sweden) as described by Boyum (1968). The collected cells were washed thrice and suspended at a concentration of 2×10^5 cells/ml in Eagle's minimum essential medium containing 5% fetal bovine serum (FBS), 100 units/ml of penicillin and 100 μg/ml of streptomycin. The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 until use. Viability was assessed by trypan blue (1%) exclusion.

Sheep blood was obtained from the Faculty of Veterinary Medicine (Ahmadu Bello University, Zaria, Nigeria) by bleeding through the jugular vein. The blood was collected in Alsevers solutions (1:1). Erythrocytes were washed three times with PBS and suspended at 1% (v/v) in the same buffer.

Triplicate aliquots (0.2 ml) of lymphocytes in one reactive mixture were mixed v/v with the sheep red blood cell (SRBC) suspension and incubated at 37°C for 30 min, centrifuged (1000 × *g*, 5 min) and reincubated for 1 h at 4°C. The superna-

tant fluid was aspirated and slides made from the cell pellets with 1% trypan blue added to distinguish between live and dead cells.

The percentage of rosetting viable lymphocytes was counted under the microscope.

For HAI assays venous blood (5 ml) was collected and allowed to clot at 4°C overnight. The serum was separated by centrifugation (2000 × *g*, 15 min) and stored at −20°C (with 2 drops of 1% Azide drops added as preservative) until use.

Rosen's HAI method (1961) was employed with minor modifications. The sera were pretreated with 25% kaolin suspension (w/v) for 20 min at room temperature, to remove non-specific inhibitors of haemagglutination. Serial two-fold dilutions (0.2 ml) of the treated serum were mixed with 0.2 ml of measles virus haemagglutinin (8 HA units/0.4 ml) and kept at room temperature for 1 h before mixing with 0.2 ml of 0.5% suspension of patas monkey red blood cells. Such cells were used within 1 week of preparation. Titers were read after incubation at 37°C for 4 h.

The results of rosetting lymphocyte counts and of HAI assays are given in Table 1. Before vaccination, HAI-antibodies were undetectable in all but one of the 5 monkeys. Rosetting lymphocyte counts were low (7.27 ± 2.34). After vaccination, each of the monkeys had detectable HAI antibody. In addition, rosetting lymphocyte counts were elevated in each animal (average of 27.07 ± 8.47). As tested by *t*-test for paired values, this difference is statistically significant ($P < 9.25$). Furthermore, a good correlation ($r = 0.96$) existed between HAI-antibody titers and rosetting lymphocyte counts. The correlation coefficient ($r = 0.96$), assuming the data came from a normal population was significant after vaccination, while prior to vaccination the correlation coefficient ($r = 0.23$) was not significantly different from zero ($P > 25\%$). Thus, there was no correlation between rosette and HAI titer pre-vaccination, while there was a good correlation post-vaccination.

TABLE 1

Number of rosetting lymphocytes and HAI antibody titers before and after measles virus vaccination in monkeys

	Monkey No.	Rosetting lymphocyte count (% ± SE; assay in triplicate)	HAI-antibody titer (inverse of end-point dilution)
Before vaccination	1	8.3 ± 1.45	< 2
	2	6.7 ± 1.30	2
	3	7.0 ± 1.15	< 2
	4	9.0 ± 1.53	< 2
	5	5.3 ± 0.88	< 2
After vaccination	1	33.3 ± 3.18	256
	2	21.7 ± 1.77	64
	3	17.7 ± 1.45	16
	4	37.3 ± 3.76	256
	5	25.3 ± 2.33	64

Our results are in agreement with the work of Utermohlen et al. (1978) which demonstrated a poor rosette formation in multiple sclerosis patients showing depressed CMI repsonses to measles antigens in comparison with normal individuals.

High numbers of avid rosette cells are also found in other conditions such as auto-immune diseases and infections with enveloped viruses other than measles virus (Dore-Duffy and Zurier, 1979). However, the significant correlation, demonstrated in this study, with high HAI antibody titers suggests that enumeration of rosetting lymphocytes may be useful to monitor development of CMI to measles virus.

The E-rosette formation is easily performed and has certain advantages over other in vitro techniques to monitor CMI, namely simplicity, low cost and greater capacity. These advantages may facilitate large-scale epidemiological studies, as well as the evaluation of vaccination programmes. These attributes make the technique potentially more suitable for monitoring measles infection and success of vaccination programmes in developing countries.

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