

Cellular Immunity in Dengue Infections

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- OBJECTIVE:** 1) To develop an in vitro system to measure cell mediated Immunity (CMI)
- 2) To determine whether or not CMI to dengue virus appears in primates and humans following dengue infection.

BACKGROUND: Little is known of the processes which confer lasting and effective immunity to arthropod-borne viruses. Measurement of any single parameter of the immunological response, such as circulating antibody, may not correlate with resistance to disease under all circumstances. There is increasing evidence that lymphocyte mediated cellular immunity (CMI) confers protection against virus diseases caused by paramyxo, herpes, pox and certain oncogenic viruses. Conversely, in some virus infections, it is likely that CMI may actually participate in the pathogenesis of illness. There have been no published studies of the development and function of lymphocyte mediated CMI in arbovirus infections of man or primates. This project was designed to test for the presence of CMI in arbovirus infections of these hosts using newly developed in vitro techniques.

PROGRESS: Work on this project started several years ago by COL Thomas J. Smith and Dr. Ananda Nisalak, who introduced the macrophage inhibition assay for macrophage inhibition factor (MIF) into this laboratory. The current investigators have attempted to refine this highly specific test for cell mediated immunity and adapt it to the requirements of the project.

Briefly the technique is based on the observation that lymphocytes obtained from immunized donors, incubated in vitro with the immunogen, (antigen) excrete a factor (MIF) which inhibits migration of macrophages out of capillary tubes. In the method employed, peritoneal exudate cells from adult guinea pigs are allowed to migrate from capillary tubes onto cover slips in small culture (Mackness) chambers. MIF is considered present in the culture medium when the measured area of macrophage migration in "MIF" medium is less than 80% of the area measured in the presence of control media not containing MIF. Table 1 describes the 4 media used in each MIF assay.

First a number of different culture media were tested for suitability for sustaining migration of guinea pig exudate cells (macrophages) from capillary tubes. The most suitable medium consisted of Medium 199, 0.1% glutamine, 7% NaHCO₃, 10% inactivated guinea pig serum, 5% fetal bovine serum, antibiotics, and 5% CO₂.

The migration of macrophages obtained from female and male guinea pigs was next compared. The area of macrophage outgrowth from the cut end of capillary tubes was photographed at 24 & 48 hrs, the photograph traced, and the area under the tracing measured with a planimeter. The average area of cell migration for 7 male guinea pigs was 1.71 cm² and for 7 females, 1.40 cm². Males were therefore selected as macrophage donors.

Next studied was the inherent error and reproducibility of the MIF technique. With the collaboration of LTC R.A. Grossman, Dept. of Epidemiology, a statistical evaluation was made of macrophage outgrowth, looking for possible sources of error. The design of this experiment involved testing macrophages taken

from 3 guinea pigs (4 tubes per animal), with area measurements made at 24 & 48 hours. The data was analyzed by the factorial statistical analysis. Our conclusions were 1) repeated measurements of the same tracing are fairly precise ($\pm 5\%$). 2) differences existed between the 3 animals used, although in this particular test they were reflected only in differences in the variability of the observations on capillary tubes and not of their means. Variability found was principally due to the inclusion of outgrowth patterns which were difficult to trace and it was concluded that such tracings should be eliminated. Since occasionally 1 or even 2 of the capillary tubes show unexplained irregularly contoured or incomplete outgrowth patterns, at least 4 tubes per test were required. 3) Tracings at 24 hours were as reliable as tracings at 48 hrs.

We next attempted to demonstrate release of MIF by lymphocytes taken from PPD skin test positive donors. Lymphocyte purified to $>90\%$ on glass bead columns, and mixed leukocytes containing 50% lymphocytes were incubated with PPD (40 ug/ml) for 3 days. Two "MIF" media were examined; one was not concentrated, and the second was concentrated to 1/5 the original volume on an Amicon ultrafiltration unit and then incubated with macrophages in capillary tubes. Macrophages were similarly incubated with concentrated or unconcentrated control media (see Table 1 for basic experimental design). No inhibition of macrophage migration was observed on several attempts. Unsuccessful experimental variations were attempted: 1) use of killed Mycobacterium tuberculosis instead of PPD, 2) mixing human buffy coat cells with guinea pig macrophages in the capillary tube and then adding PPD antigen to this culture medium, 3) adding lymphocytes and antigen directly to the culture medium bathing the capillary tubes. In addition we incubated streptokinase—streptodornase (SK—SD) antigen (50 units) with lymphocytes ($3-5 \times 10^6$ cells/ml) taken from a SK—SD skin test positive donor. One—half of the medium was withdrawn everyday, and fresh antigen containing medium added. The medium was pooled, concentrated 5 to 10 fold, and incubated together with suitable control media within capillary tubes in Mackness chambers. On 2 occasions inhibition of migration (25% and 50%) was noted in the immune lymphocyte and antigen preparations but in both experiments one of the 3 control preparations also produced inhibition of migration. A third attempt showed no inhibition of migration by the immune or control preparations. Further attempts to demonstrate release of MIF have been indefinitely postponed. Attention will now focus on the less specific, but technically easier lymphocyte transformation technique. Whatever in vivo CMI technique is finally selected, it must work with no more than 10—15 ml whole blood, which is the maximum volume of blood that can be drawn from children or primates at any one time.

Table 1.
Media used for MIF assay

Medium	Culture medium incubated 3 days with:	
	Lymphocytes	Antigen
1. "MIF"	+	+
2. Control	+	0 ^{xx}
3. Control	0	+
4. Control	0	0

* + = present in culture medium
xx 0 = not present in culture medium