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Improvements to the Plaque Assay for Antibody Secreting Cells

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A capillary glass microslide has been adapted to function as a chamber for the determination of plaque forming cell responses. Comparisons with Cunningham chambers indicate no significant difference between methods. Further, microslides arrive clean and ready to use, thus eliminating the need to assemble chambers as necessitated by the Cunningham method. Used in conjunction with an electronically assisted enumerating device, the microslides provide a rapid and less tedious means for assaying large numbers of animals for PFC responses.

Key words *capillary glass microslide – Cunningham chambers – plaque forming cell response*

Introduction

The plaque forming cell (PFC) response of mice challenged with heterologous erythrocytes is one of the most studied phenomena in the field of immunobiology. The initial description of this methodology was published by Jerne and Nordin (1963), and modified versions of the PFC assay have appeared and come into more general acceptance. One assay modification reported by Mishell and Dutton (1967) was prompted by their interest in studying PFC responses generated in vitro. This method, requires fewer numbers of cells to assay a hemolytic response, utilizes microscope slides rather than more cumbersome petri dishes for plaque development and offers the advantage that the plaques can either be scored immediately after development or they can be stored overnight at 4°C and scored the next day. On the other hand, this method has the disadvantage of requiring labor-intensive gelatin coating of microscope slides before use. To ensure the necessary homogeneity of the reaction mixture, both methods expose indicator red cells and antibody-secreting

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cells to elevated temperatures (45°C) to ensure liquification of the agarose matrix.

Another modification of the plaque assay developed by Cunningham (Cunningham, 1965; Cunningham and Szenberg, 1968) calls for the development of plaques in a liquid monolayer of indicator red cells. The monolayer is formed between 2 microscope slides separated by thin double-sided adhesive tape. This modification offers the advantage of increased resolution and sensitivity and avoids the elevated temperature of the Jerne and Mishell–Dutton procedures. Disadvantages of this method arise from the absolute requirement for scrupulously clean microscope slides to ensure that no voids develop during chamber filling and from the considerable time that must be expended in assembling the chambers.

While all of the above methods are hampered by the labor-intensive nature of the assay, a more worrisome problem is the subjectivity inherent in enumerating plaques. Using an electronically assisted scoring system, Katz et al. (1977) seem to have acquired more objectivity by electronically evaluating changes in optical density occurring when a small area of indicator red blood cells were lysed.

We describe here a method for PFC quantitation which minimizes both the labor intensive requirements and the inherent subjectivity of previously described systems. The difficulties, the slide washing and the cost of the glass slides are avoided by using glass capillary microslides. The desired objectivity was achieved by using an electronically assisted enumeration system. Results obtained with this method were reproducible and comparable to those obtained using the Cunningham chamber method.

Materials and Methods

Capillary microslides

Glass microslides (Vitro-Dynamics, Rockaway, NJ 07866) were of the dimension 0.22 mm × 4 mm × 100 mm (special order) yielding a capacity of 88 μl. These dimensions reduced the problem of bubbles that occasionally appeared in the chambers. The microslides were boiled in distilled water prior to shipment and arrived clean and ready-to-use.

Preparation of Cunningham chambers

Cunningham chambers were made according to the method of Cunningham and Szenberg (1968) using Gold-Seal pre-cleaned microscope slides (Clay-Adams) and Scotch no. 410, 1/4 inch width double sided adhesive tape. The microscope slides were soaked for a minimum of 1/2 h in 3 N HNO_3 rinsed with copious amounts of distilled water and allowed to dry. Dual chambers were created using 3 pieces of tape yielding a final volume of approximately 100 μl per chamber.

Mice strains and immunization method

Mouse strains used were C57BL/6J, CBA/J, C3H/HeJ (Jackson Labs.). Mice were immunized with 200 μl of 10% sheep red blood cells (Gibco) in 0.9% NaCl injected into the intraperitoneal cavity on day 0. Mice were killed and spleens were

removed on day 5 unless otherwise noted. Spleen cells were prepared according to the method of Mishell and Shiigi (1980).

PFC development

The reaction mixtures consisted of: 25 μl SRBC at 12.5%, 25 μl guinea pig complement (1 : 2), 100 μl murine spleen cell suspension at appropriate concentrations, 100 μl RPMI 1640 with Hepes and 5% fetal bovine serum. All reagents were diluted to indicated concentrations with RPMI 1640/5% FBS with 15 mM Hepes. All reagents were combined in individual polystyrene micro-titer wells (Costar). After reagents were combined and thoroughly mixed, they were pipetted with Pasteur pipettes into Cunningham chambers. In the case of microslides, one of the ends was placed into the reaction mixture and readily filled by capillary action. Both Cunningham chambers and microslides were sealed with a paraffin-petrolatum mixture and incubated 1–2 h at 37°C.

Plaque counting

Microslides as well as Cunningham chambers can easily be counted visually under a low power microscope. For greater objectivity and speed, the Artek Systems Counter Model 980 (Artek Systems, Farmingdale, NY 11735) was used to score plaques. This instrument has demonstrated applications for PFC counting (Katz et al., 1977). The instrument may be used with or without the electronic counting mode. Briefly, the Artek counter relies upon the difference of optical density between the plaque and the surrounding red blood cell monolayer. This difference in optical density is processed electronically through the instrument and each plaque is 'flagged' on the monitor, thereby assuring the user that the plaques are being correctly enumerated. Values of PFC/10^6 spleen cells were determined according to the formula:

$$\frac{\text{total volume of reaction mixture}}{\text{chamber volume}} \times \text{dilution factor} \times \text{PFC/slide.}$$

Optimal number of plaques is in the range of 30–50 per slide, although greater numbers have been counted without resultant loss of resolution.

Statistics

Statistical analyses were done with arithmetic mean and standard error (SE) calculated from individual values in each group. Student's *t*-test was used for comparison of microslide PFC versus Cunningham chamber PFC within an experiment while 2-way analysis of variance was used to test significance in kinetic studies where data were accumulated in separate experiments.

Results

A comparison of microslide and Cunningham chamber data is shown in Table I. In each case, the number of PFCs in the total area was counted.

TABLE I

COMPARISON OF DATA OBTAINED FROM MICROSLIDES AND CUNNINGHAM CHAMBERS

Student's *t*-test indicates no significant difference between microslides and Cunningham chambers, $P \leq 0.05$

Microslide		Cunningham chamber	
Replicate no.	PFC/10^6 spleen cells	Replicate no.	PFC/10^6 spleen cells
1	1250	1	1330
2	1344	2	1420
3	1000	3	1200
4	906	4	790
5	1134	5	1120
6	1063	6	1310
7	1281	7	1210
8	1134	8	1300
9	1406	9	1430
10	1031	10	1360
11	1594	11	1440
12	1719	12	1290
13	1343	13	1340
14	1281	14	1430
		15	1410
$\bar{x}$ = 1249.0		$\bar{x}$ = 1292.0	
SD = 228.2		SD = 168.0	
SE = 60.99		SE = 43.38	

As can be seen, the difference of the means between the 2 methods was 3%, an insignificant difference. The range of PFC/10^6 spleen cells for the microslides was 813 PFC; i.e., from 906 to 1719. In the case of the Cunningham chambers, the range

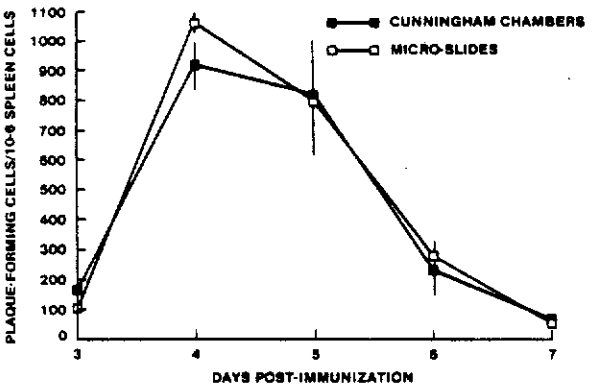

Fig. 1. Time course study of plaque forming cell response. Data of Cunningham chambers are the sum of dual chamber slides. On day 0 all mice received 200 µl of 10% SRBC in 0.9% NaCl. Data of each day represent 5 mice/group. Shown are the mean values ± S.E. The spleens were pooled and the PFC assay was run in triplicate. CBA/J mice response is shown.

TABLE II

DAY-TO-DAY REPRODUCIBILITY OF PFC ESTIMATION USING MICROSLIDES

Group	n	PFC/spleen (mean)	SE
1	8	136746	22442
2	7	138876	12328
3	8	141006	14070
4	8	147396	18125
5	8	148248	16849

was 650 PFC/10^6 spleen cells, i.e., from 790 cells to 1440. These data were obtained with C57BL/6J mice and plaques were scored using the Artek Systems Counter Model 980.

Kinetic studies data for 2 strains of mice are illustrated in Fig. 1. While differences between PFC/10^6 spleen cells are visible, they proved to be inconsequential when subjected to a 2-way analysis of variance and were well within the parameters of the null hypothesis.

A third study was undertaken to determine the reliability of the microslides. In this experiment, 40 CBA/J mice were divided equally into 5 groups and 300 μl of a 7% SRBC suspension in 0.9% NaCl was injected i.p. into 1 group per day for 5

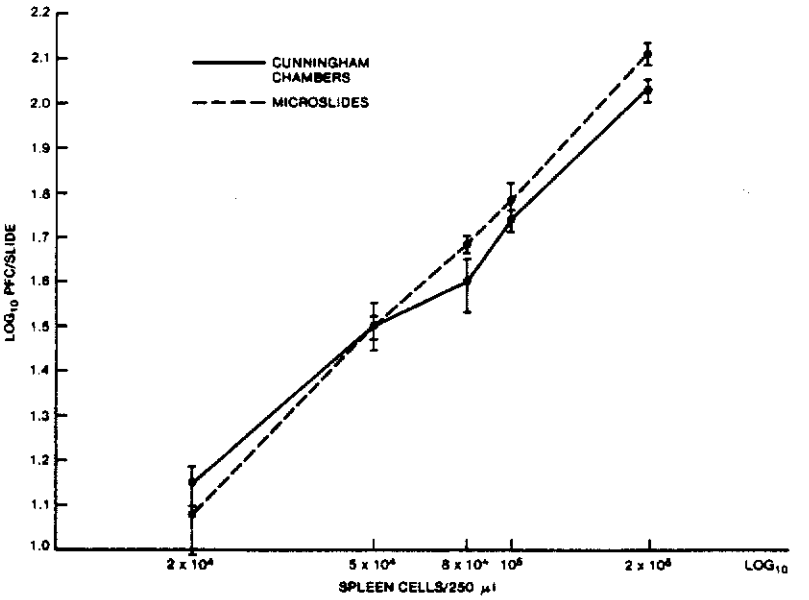

Fig. 2. The effect of incremental dilutions of spleen cells from the same spleen cell preparations on number of PFC/slide. Data represent mean ± SEM of 6 microslides and 3 dual chamber Cunningham slides. Mice received 3×10^8 SRBC in 0.5 ml of 0.9% NaCl i.p. All dilutions of spleen cells were obtained from single spleen cell preparations.

consecutive days. Plaque-forming cells were enumerated in each group 4 days after antigen administration. The range of the means between group 1 and group 5 is 11502 PFC/spleen, well within the standard error of the mean of the individual groups (Table II). Thus, the reproducibility of microslides from day to day was established.

An examination of the effect of incremental dilutions of spleen cells from the same spleen cell preparation on the quantitative relationship between Cunningham chambers and microslides is illustrated in Fig. 2. The spleen cell concentrations examined were: 2×10^4, 5×10^4, 8×10^4, 1×10^5 and 2×10^5 spleen cells/250 μl. The PFC determinations resulting from the 2 types of chambers were then normalized for volume differences. As shown in Fig. 2, both the Cunningham chambers and the microslides generated data points which produced a straight line when plotted as a function of the cell concentrations tested.

Discussion

The microslide method clearly compares favorably with the Cunningham slide technique in terms of quantitation and reliability. In addition, microslides offer several advantages. They arrive pre-cleaned and ready-to-use. No pipetting is necessary, as merely placing one end into the microtiter well causes the slide to fill by capillary action. The microslide volumes are uniform, thereby enhancing plaque-counting reproducibility. The plaque size seen in the microslides are comparable to those in Cunningham chambers. Data have not been obtained with either haptenated SRBC or indirect plaque assay methods; however, there is every reason to expect that microslides would be adaptable to these procedures. Finally, when microslides are used in conjunction with an automated counter such as the Artek Systems Counter Model 980, they provide a rapid, objective and less tedious means of assaying large numbers of animals for plaque forming cell response.

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