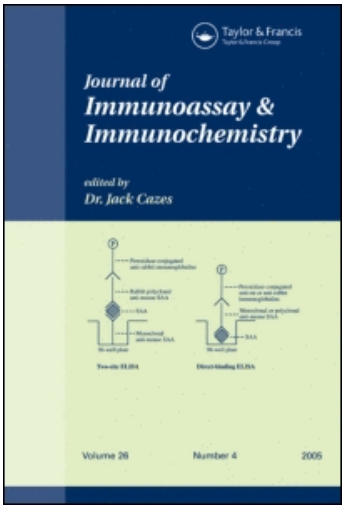

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SYNTHESIS OF HUMAN IMMUNOGLOBULINS IN VITRO: COMPARISON OF TWO ASSAYS OF SECRETED IMMUNOGLOBULIN

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ABSTRACT

One consequence of B-lymphocyte activation is immunoglobulin production, which can be quantitated by various techniques. We have compared assay of plaque forming cell (PFC) and determination of immunoglobulins by ELISA in culture supernatants of human lymphocytes stimulated with pokeweed mitogen and with Staphylococcus aureus. These assays correlated well ($r > 0.77$) in all major immunoglobulin classes studied.

The close correlation suggests that determination of secreted immunoglobulins by ELISA may be substituted for the PFC assay.

(KEY WORDS: Immunoglobulin synthesis in vitro)

INTRODUCTION

Activation of B-lymphocytes results in production of antibodies. In vitro this can be measured 1) by counting the cells with intracytoplasmic immunoglobulins (1,2), 2) by counting the cells secreting immunoglobulins with a plaque forming cell assay (PFC) (3,4,5) or 3) by determining immunoglobulins secreted into the culture medium. The latter can be performed with sensitive techniques like enzyme-linked immunosorbent assay (ELISA) (6,7), radioimmunoassay (8,9,10,11) or nephelometry (12). These methods for assessing B-lymphocyte function differ considerably in ease of performance, accuracy, and scope for observer errors.

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Each method may measure different aspects of B cell function and it has been claimed that measurements of PFC and immunoglobulins do not correlate (12,13). Our earlier work on in vitro antibody synthesis suggested that PFC assay and immunoglobulin determinations might indeed give similar results (14). The present study was therefore conducted to compare systematically these techniques and to determine whether the PFC assay could be replaced by ELISA, a method with many practical advantages.

MATERIAL AND METHODS

Cell Suspensions

Peripheral blood mononuclear cells from twelve healthy donors were separated by Ficoll-Isopaque centrifugation (15) and washed twice with Hanks balanced salt solution (HBSS). In order to detach the cytophilic antibodies the cells were incubated in HBSS at 37°C for 30 min. Thereafter the cells were washed once with HBSS and resuspended in RPMI 1640 (Flow Laboratories Ltd., Irwine, Scotland) supplemented with 10 mmol/l HEPES, gentamicin 70 mg/L (Schering Corp. Kenilworth, USA) and L-glutamine 0.3 g/L, and 10% heat inactivated fetal calf serum (FCS, Flow Laboratories). The number of cells was adjusted to 10^9/L.

Mitogens and Cell Cultures

Duplicate cell cultures were set up in 16x100 mm culture grade round bottomed plastic tubes (Sterilin Ltd.,Teddington, England) in a volume of 1 ml with or without pokeweed mitogen (PWM, Gibco Co. New York, USA) 1:100 final dilution, or Staphylococcus aureus Cowan I 0.05v/v% (16). The cultures were incubated for 6 days in a humid atmosphere of 5% CO_2 and 95% air at 37°C. After incubation the duplicate cultures were pooled and the cells pelleted. The supernatants were stored at -20°C until assayed by ELISA.

Secondary Cultures

On day 6 the cells from the initial cultures were washed with HBSS and suspended in 2 ml of medium without FCS. A 1 ml aliquot of the

suspension was used for PFC assay and the rest incubated for 20 hours at 37°C. The supernatants were made cell free and frozen.

Plaque Assay

The number of Ig-secreting cells was determined by haemolytic plaque assay (5,14) using sheep red blood cells coupled to Protein-A by chromic chloride method (17).

Enzyme-Linked Immunosorbent Assay

The concentration of immunoglobulins in culture supernatants was determined by microplate ELISA. A double antibody sandwich method for quantitation of IgA, IgG and IgM was used (7,14). The antisera and alkaline phoshatase conjugates were from Orion Diagnostica, Helsinki, Finland.

RESULTS

Long Term Cultures

Time course studies have shown that in our lymphocyte cultures plaque forming cell maximum is on day six or seven, although the concentration of secreted immunoglobulins in the medium increases further beyond this time (data not shown). Supernatants were collected on the sixth day of culture and PFC were assayed. Fig.1 shows PFC/culture plotted against the concentration of immunoglobulin measured by ELISA in the corresponding culture supernatants. PFC calculated per 10^6 viable cells were similar as PFC calculated per culture. The plot contains all cultures regardless of the stimulant used. The range of PFC/culture was for IgM: 135-29,200, for IgG: 270-60,600 and for IgA: 60-22,000. Immunoglobulin concentrations ranged for IgM: 23-13,500 µg/L, for IgG: 89-12,000 µg/L and for IgA: 36- 2,300 µg/L.

Secondary Cultures

We expected that, due to timing of secondary cultures, even closer correlation would exist between the amount of secreted

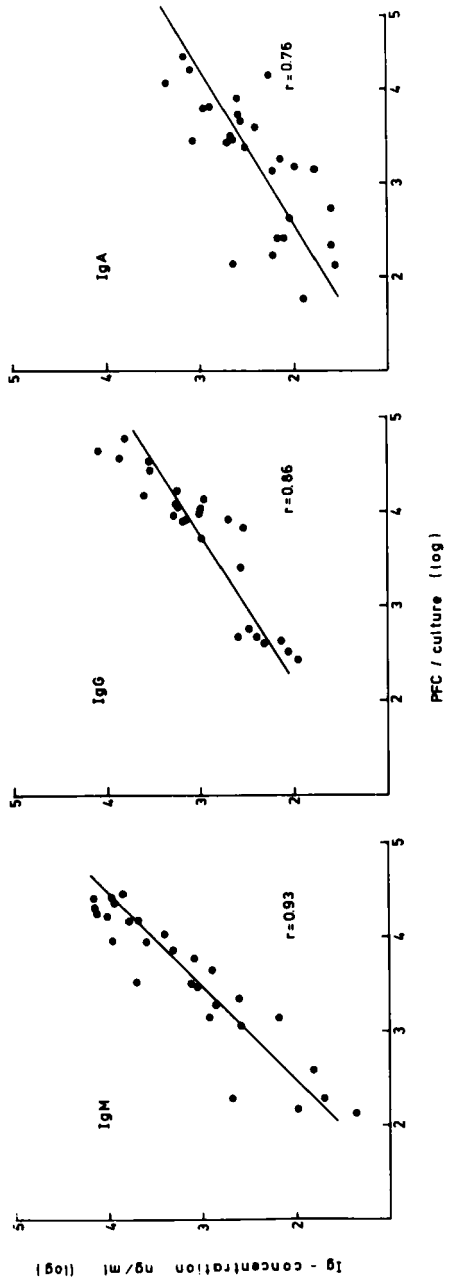

FIGURE 1. Correlation of PFC with concentration of secreted immunoglobulins in six day cultures ($p < 0.001$).

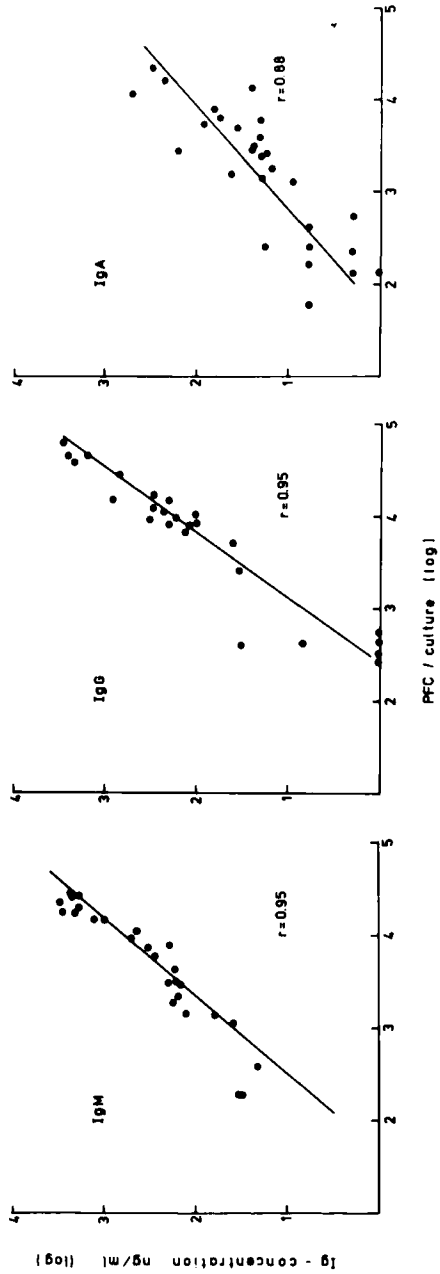

FIGURE 2. Correlation of PFC with concentration of immunoglobulins in secondary short term culture supernatants ($p < 0.001$).

immunoglobulins in those supernatants and the number of PFC. Fig.2 is a plot of the number of PFC/culture against the concentration of immunoglobulins in a 20 h culture set up at the time of the PFC assay. The concentrations of immunoglobulins were, IgM: 0-2,870 µg/L, IgG: 0-3,000 µg/L and IgA: 0-500 µg/L.

DISCUSSION

The purpose of this study was to demonstrate whether the PFC assay can be replaced by measurement of immunoglobulin concentration in culture media in studies of *in vitro* immunoglobulin synthesis. We found that the concentrations in both the short and long term culture supernatants correlate well with the number of PFC. Other workers have reported an absence of correlation (12,13). It is unlikely, however, that these differences can be explained by differing PFC assay techniques (20). There may have been different kinetics of cellular events or differences of sample timing between the laboratories. It has been shown (18,19) that before day 4 virtually no PFC are found. The number of PFC reaches its maximum around day 6 and thereafter decreases rapidly. During that time immunoglobulins accumulate in the culture supernatant and the concentration is still increasing when the number of PFC is already decreasing. It is therefore clear that PFC and immunoglobulin concentrations can not correlate after the PFC peak. In the present study the comparisons were made before or at the peak.

ELISA overcomes many problems associated with PFC assay. The difficulties with target SRBC and antibody-mediated complement lysis are avoided. Supernatant samples can be stored for batch assay and can be reassayed Furthermore the measurement is objective and easily automated. When there are, however, only few immunoglobulin secreting cells, ELISA is not sensitive enough and PFC assay can be more useful. In addition, no costly instruments are needed for the PFC assay.

The present results suggest that assay of secreted immunoglobulins may be used as an alternative to PFC assay. This study was, however, limited to healthy individuals and it is possible that cells from patients with immune abnormalities would behave differently. It is also not certain that all the stimulants will activate B cells in the same way as PWM or Staphylococcus aureus used in this study. Furthermore, immunoglobulin subclass production may have variable effects on the PFC assay and ELISA. These points are presently under investigation.

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