

## CHEMILUMINESCENCE RESPONSE IN NORMAL HUMAN PHAGOCYTES. II. EFFECT OF PARAPROTEINS

J. P. ROBINSON and R. PENNY

*Department of Immunology, St. Vincent's Hospital, Sydney, Australia and Department of Medicine, University of N.S.W., Sydney, Australia*

(Received 22 June 1981)

**SUMMARY** We have used chemiluminescence (CL) as a model for assessing the effects of paraproteins on human neutrophils and monocytes. A difference in the response of these two cell types was demonstrated. Paraprotein-containing sera (classes IgG, IgA and IgM) depressed the CL response of monocytes in almost all cases but had no effect on neutrophils. This effect was reproduced using purified paraprotein and showed a dose dependent response. It is proposed that this difference may be a function of the interaction between paraprotein and differing membrane receptors in phagocytes.

### INTRODUCTION

PARAPROTEINS have been shown to have a variety of effects on neutrophil function. This effect probably occurs as a result of a paraprotein interaction with the neutrophil (PMN) membrane. Phagocytosis is reduced, as is the adhesiveness and skin window response (1-3) however both suppression (4) and enhancement (5) have been demonstrated on chemotactic studies. Most patients with paraproteinaemia who also have phagocytic defects have serious and recurrent infections (3). With respect to monocyte (MN) function, studies in patients with haemolytic malignancies have demonstrated increased MN chemiluminescence (CL), although no changes were observed with solid tumours (6). The chemiluminescence response of phagocytes after metabolic activation is a consequence of that activation, and therefore the measurement of CL can be utilized as a corresponding measure of leukocyte function (7-9). It is known that the addition of increased concentration of isolated paraprotein to normal PMN produces a progressive reduction in phagocytic capacity. Because of this selective effect it is assumed that there exists some specificity in the interaction of paraproteins with the PMN membrane. In order to examine the activation of both PMN and MN in paraproteinaemia we utilized the CL assay as an index of phagocyte response. CL responses of PMN and MN were measured in the presence of both paraprotein containing serum and isolated purified paraproteins.

*Correspondence:* Ronald Penny, Associate Professor of Medicine, Department of Immunology, St. Vincent's Hospital, Darlinghurst, N.S.W. 2010, Australia.

### MATERIALS AND METHODS

#### *Preparation of PMN and MN*

Peripheral blood phagocytes were obtained from healthy volunteers and were isolated by Ficoll-Paque separation followed by dextran sedimentation. 10 ml heparinized blood was diluted 1:2 with phosphate buffered saline (PBS) and then carefully layered onto 10 ml Ficoll-Paque in 50 ml centrifuge tubes (Corning, USA). These tubes were centrifuged at 1200 g for 30 minutes after which the upper cell layer was removed and washed with Hanks' balanced salt solution (HBSS) ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  free), containing 15% Fetal Calf Serum (FCS). This MN containing layer was then washed twice with HBSS ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  free FCS). This was followed with a final wash with normal HBSS. Cells were then resuspended in HBSS at a concentration of  $1 \times 10^6$  Mn/ml. The PMN rich layer was sedimented with 3% Dextran (Pharmacia) for 45 minutes followed by removal of the PMN fraction. PMN were then spun at 200 g followed by hypotonic lysis of remaining red blood cells. PMN were then washed three times in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  free PBS containing 15% FCS. This was followed by one wash with normal HBSS. PMN suspensions used in each assay were 99% pure.

#### *Zymosan Preparations*

500 mg zymosan A (Sigma) was boiled in PBS for 1 hour, washed and resuspended in PBS at 50 mg/ml. 1 ml of zymosan solution (50 mg) was opsonized with 1 ml serum and 1 ml PBS for 30 minutes at 37°C, by rotating the tubes at 12 RPM on a multi purpose rotator (Scientific Industries, Springfield, Mas.). The zymosan was then washed and resuspended in PBS at a concentration of 5 mg/ml. 1 ml was then added to phagocytic cells.

#### *Luminol Preparation*

10 mg luminol (Sigma) was dissolved in 1 ml of dimethyl sulphoxide and 100 ml PBS. This was diluted to 1 part luminol per 100 ml PBS and 100  $\mu\text{l}$  added to each vial.

#### *CL Measurements*

CL measurements were made on a Packard 2660 Liquid Scintillation Counter (Packard Instrument Co.) as reported (10). Briefly, a series of 10 vials were counted repeatedly at 2.5 minute intervals over 60

minutes. Peak CL responses were then recorded for each vial. All assays were carried out at 24°C.

*Paraprotein Sera*

A total of 31 different paraprotein sera were tested in this study (15 IgG, 10 IgA, 6 IgM). These sera had been either stored at -20°C or were fresh samples. When whole paraprotein containing sera were tested, a total of 500 µl was added to vials to produce final concentration of serum of 15%.

*Isolation of Paraproteins*

Isolation of IgG paraproteins was achieved by protein A affinity chromatography and IgM paraprotein by means of Sephadex G200 gel filtration. The purity of the immunoglobulin preparations was confirmed by immunoelectrophoresis (11) and immunodiffusion (12) using polyvalent and class specific immunoglobulin antisera. Paraprotein concentrations used were in the range of 20-60 mg/ml.

*Control Sera*

Control sera were taken from healthy laboratory staff and were either stored at -20°C or were fresh samples.

**RESULTS**

With control PMN no significant difference was found between the level of peak response of CL in the presence of the 31 paraprotein-containing sera (15 IgG, 10 IgA, 6 IgM) compared with control human serum (fig. 1). With MN a marked reduction in peak CL response was found with 25/29 paraprotein-containing sera (12/14 IgG, 8/10 IgA, 5/5 IgM) compared with control human serum ( $p < 0.01$ ) (fig. 2). Although peak responses were reduced, the time to reach these peaks was unaltered. With PMN peak response occurred at 22 minutes whereas for MN this peak was 14 minutes after the addition of zymosan. When isolated paraproteins were used no significant changes were observed in peak CL response of control PMN in

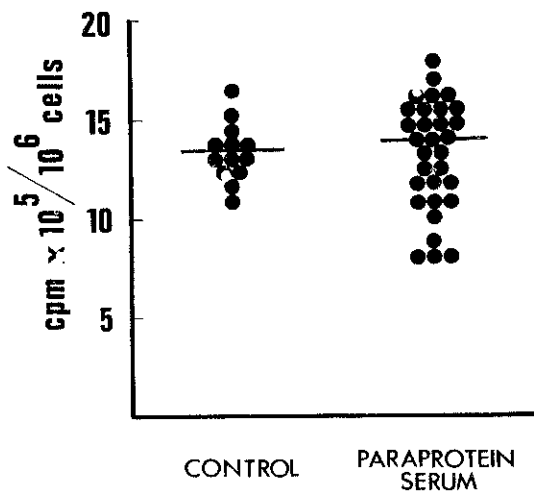


FIG. 1. Peak CL response of normal neutrophils in the presence of paraprotein containing serum (15 IgG, 10 IgA, 6 IgM). Bars indicate mean.

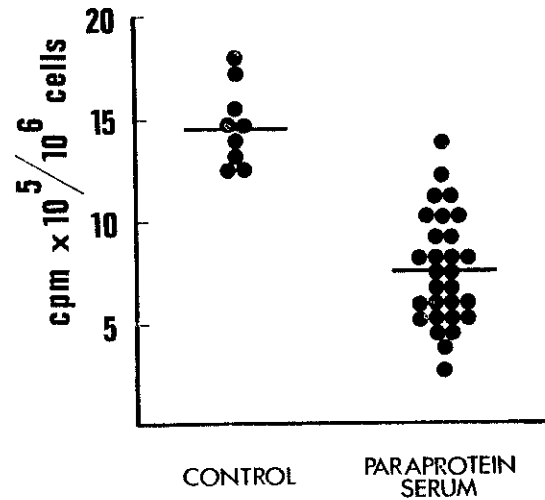


FIG. 2. Peak CL response of normal monocytes in the presence of paraprotein containing serum (14 IgG, 10 IgA, 5 IgM). Bars indicate mean.

the presence of either 25 or 50 mg of two different IgG paraproteins. However, one IgM paraprotein significantly reduced the peak CL response of control PMN as shown in Figure 3. In the presence of 10 mg of this paraprotein, an 83% reduction in peak CL was shown and a 95% reduction in the presence of 25 mg paraprotein.

There was a marked dose dependent reduction of peak CL responses of normal MN by 3 different IgG paraproteins (see fig. 4). In the presence of 25 mg of paraprotein the 3 IgG paraproteins caused an average decrease in peak CL of 47% and in the presence of 50 mg paraprotein the average decrease in peak CL was 76%.

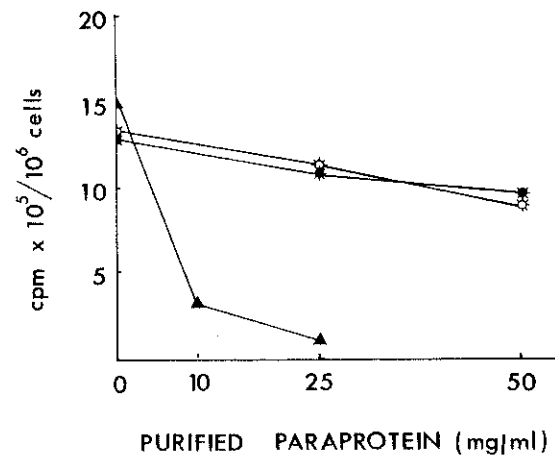


FIG. 3. Peak CL response of normal neutrophils in the presence of isolated paraprotein.  
 ○—○ IgG paraprotein (patient MF); ●—● IgG paraprotein (patient WD); ▲—▲ IgM paraprotein (patient CP).

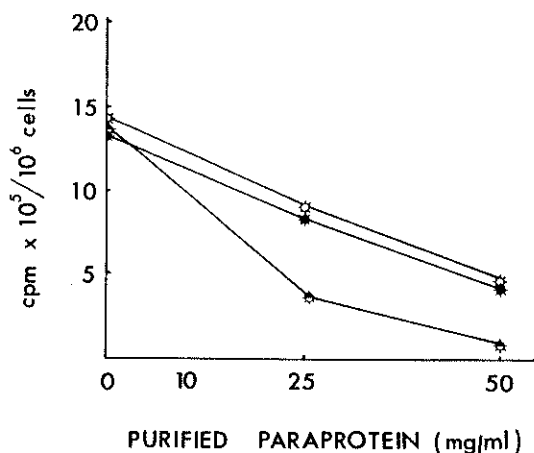


Fig. 4. Peak CL response of normal monocytes in the presence of isolated paraprotein.

○—○ IgG paraprotein (patient MF); ●—● IgG paraprotein (patient WD); ■—■ IgG paraprotein (patient RMcM).

## DISCUSSION

Previous work has demonstrated a functional impairment of neutrophil phagocytic cells in the presence of high levels of circulating paraproteins found in patients with multiple myeloma (3). We have shown a significant impairment of MN-CL response in the presence of paraproteins containing sera of class IgG, IgM and IgA. MN-CL responses were also significantly impaired in the presence of isolated IgG paraproteins. PMN CL responses were not significantly altered in the presence of paraprotein-containing sera. However, one isolated paraprotein of class IgM did cause a gross reduction in the CL response of normal PMN.

Since the CL response is a result of membrane activation it would seem that the paraproteins at high concentrations (25–50 mg/ml) interfere with availability of cell receptors for the initiation of phagocytosis. IgA paraprotein has been shown to significantly suppress chemotaxis (4). Other studies however have demonstrated enhancement (5). No explanation can be offered regarding the reduced CL response of PMN to only one IgM paraprotein while the response in the presence of IgG paraproteins was unaltered. It is possible that this difference may be due to the difference in the assay procedure used in that CL is only measuring the production of oxygen radicals. The absence of effect upon PMN CL response in the case of IgG paraprotein was

entirely unexpected in the light of previous work. The data presented indicates that there is a considerable difference in the CL responsiveness of MN and PMN in the presence of paraproteins. This difference may be a function of the interactions between paraproteins and different membrane receptors.

Other studies have demonstrated abnormal monocyte function in patients with multiple myeloma (13). The data presented indicates that presence of paraproteins significantly reduces MN-CL activity. It is unlikely that this effect is a result of a zymosan paraprotein interaction since the zymosan is preopsonized with normal serum and therefore unlikely that the paraprotein would have any significant inhibitory effect on the zymosan. It is also possible that the paraprotein is able to block MN receptors either by competitive inhibitors of Fc receptors or steric hinderance in the case of C3 receptors. Further work to resolve this uncertainty is in progress.

## REFERENCES

1. Penny, R. (1969). A study of neutrophil function in health and disease. MD Thesis, University of Sydney.
2. Penny, R. and Galton, D. A. G. (1966). Studies on neutrophil function. II. Pathological aspects. *Br. J. Haematol.*, **12**, 633.
3. Penny, R. Castaldi, P. A. and Whitsed, H. M. (1971). Inflammation and haemostasis in paraproteinaemias. *Br. J. Haematol.*, **20**, 35.
4. Van Epps, D. E. and Williams, R. C. (1976). Suppression of leukocyte chemotaxis by human IgA myeloma components. *J. Exp. Med.*, **144**, 1227.
5. Robinson, J. P., Boughton, R. and Penny, R. (1979). Chemotaxis and chemiluminescence in paraproteinaemia. *Proc. Aust. Society of Immunology*, Sydney.
6. Kitahra, M., Eyre, H. J. and Hill, H. R. (1979). Monocyte functional and metabolic activity in malignant and inflammatory diseases. *J. Lab. Clin. Med.*, **93**, 472.
7. Allen, R. C., Stjernholm, R. L. and Steele, R. H. (1972). Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. *Biochem. & Biophys. Res. Com.*, **47**, 679.
8. Allen, R. C. (1977). Evaluations of serum opsonic capacity by quantitating the initial chemiluminescent response from phagocytosing polymorphonuclear leukocytes. *Inf. & Imm.*, **15**, 828.
9. Stevens, P., Winston, D. J. and Van Dyke, K. (1978). *In vitro* evaluation of opsonic and cellular granulocyte functions by luminol dependent chemiluminescence: Utility in patients with severe neutropenia and cellular deficiency states. *Inf. & Imm.*, **22**, 41.
10. Robinson, J. P. and Penny, R. (1981). Chemiluminescence response in normal human phagocytes. I. Automated measurements using a standard liquid scintillation counter. *J. Clin. Lab. Immunol.*, **7**, 215.
11. Scheidegger, J. J. (1955). Une micro-méthode de immunoelectrophoresis. *Int. Arch. Allergy Appl. Immunol.*, **7**, 103.
12. Ochterlony, O. (1958). Diffusion in gel methods for immunological analysis. *Prog. Allergy.*, **5**, 1.



Teviot Scientific Publications Ltd  
31 Montpelier Park  
Edinburgh EH10 4LX, UK

