The neutrophil chemiluminescence response to *Pneumocystis carinii* is stimulated by GM-CSF and gamma interferon

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1. SUMMARY

The interaction of *Pneumocystis carinii* purified from rat lung with human polymorphonuclear leukocytes (PMN) was studied using amplified chemiluminescence. Pre-incubation of PMN with granulocyte-macrophage stimulating factor or gamma interferon resulted in phagocyte stimulation.

2. INTRODUCTION

*Pneumocystis carinii* (PC) is an organism of uncertain taxonomic status, probably most closely related to the fungi [1]. It causes a severe pneumonia (PCP) in immunocompromised hosts. It is the commonest serious secondary infection in patients with the acquired immunodeficiency syndrome (AIDS) and established chemotherapeutic agents cause a high incidence of adverse reactions in this patient group [2].

The relative importance of the components of the immune response to PC in the healthy subject is unclear. Isolated congenital agammaglobulinaemia and congenital T cell deficiency states both predispose to PCP [3]. In AIDS the main lesion is lack of CD4 positive T\(_H\) lymphocytes, resulting in impaired cell-mediated and humoral responses, but primary defects in monocyte/macrophage function also occur [4,5]. The relative contribution of the polymorphonuclear leukocyte (PMN) is hard to assess. Although PMNs are not prominent in the inflammatory infiltrates seen in histological studies [6], these are usually of the chronic rather than the acute stage of infection. The surprising rarity of extrapulmonary infections [3], particularly before the AIDS era, may reflect the efficacy of circulating PMN.

Phagocytosis of PC by PMN and macrophages in vitro is increased by specific antibody to PC and complement [7]. The interaction of PC with both classes of phagocyte also causes chemilumi-
nescence (CL), which is a measure of respiratory-burst activity [8]; PMN CL is also stimulated by antibody to PC and complement (opsonins) [7]. AIDS patients lose antibody to PC before they develop PCP [9]; their phagocyte function may be compromised through loss of opsonising antibody. Cytokines have pleiotropic effects including phagocyte stimulation. Cloned cytokines may find a therapeutic role in AIDS and other immunocompromised patients. Considerably higher CL signals were detected with PMN than macrophages [7]. We decided to investigate the effect of two cytokines on PMN/PC interaction by using amplified CL.

3. MATERIALS AND METHODS

3.1. Parasites

PCP was induced in groups of 30 adult female Sprague-Dawley rats (Harlan OLAC), which are naturally infected by Pneumocystis carinii, by administration of betamethasone sodium phosphate 10 mg/l (Glaxo) in their drinking water. Tetracycline hydrochloride 1 g/l (Sigma) was also added to suppress secondary bacterial infection, and on the day before sacrifice the animals were given oral ciprofloxacin (Bayer) and an intraperitoneal injection of amoxycillin-clavulanic acid (Beechams), gentamicin (Lederle) and vancomycin (Eli Lilly). Bacterial counts on lung homogenates were thus minimised (less than one colony forming unit/10^5 PC). Parasites were separated from host-cell contamination by unit-gravity sedimentation [10] and then aliquots were stored in phosphate buffered saline (PBS) supplemented with 10% dimethyl sulphoxide at −70 °C until the day of use.

3.2. Polymorphonuclear leukocytes

20 ml of heparinised blood was collected from a healthy human volunteer and mixed with 10 ml Dextran 110 (Fisons) in a syringe which was then clamped vertically for 45 min to sediment most of the erythrocytes. The top layer was aspirated, cells harvested by centrifugation, then resuspended in 10 ml Boyle's solution (17 mM ammonium chloride, 140 mM tris(hydroxymethyl) aminomethane (Tris), pH 7.65) and incubated on a roller at 37 °C for 20 min to lyse residual erythrocytes. The remaining blood cells were washed twice in Hanks' buffered salt solution with N-2-hydroxymethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer added to 20 mM pH 7.3 (HH) and then resuspended in the same medium. Cells were then incubated at 37 °C with or without the addition of cytokines as detailed below. Granulocyte-macrophage colony stimulating factor (GM-CSF) (SeraLab) and interferon gamma (Ifn-g) (Sigma) were both recombinant human preparations. Phagocytes were always > 95% polymorphonuclear cells.

3.3. Chemiluminescence

Thawed PC were incubated in PBS with 0.5% polyclonal anti-PC antiserum (prepared in rats; details in [7]) and 20% freshly reconstituted guinea pig complement (SeraLab) at 37 °C for 20 min, washed in PBS twice, then resuspended in HH. 0.5 ml PMN was mixed with 0.9 ml of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione; Sigma) 0.02 mM in HH in a plastic cuvette. Baseline light output was measured at 37 °C using an LKB 1250 luminometer with a water-jacketed cuvette holder (Bio-Orbit, Turku, Finland). Opsonised PC (0.2 ml) were added to the cuvette and serial luminometer readings recorded at timed intervals. The peak CL in mV was determined from output-time curves for each experimental condition [7]. Parasite and PMN concentrations are specified for each experiment below.

4. RESULTS

Fig. 1 shows the effect of pre-incubation of PMN with various concentrations of GM-CSF for 150 min. Each cuvette contained 1.5 × 10^6 PMN; the ratio of PC:PMN = 45:1. There was a dose-dependent stimulation of CL by GM-CSF in the range 0.05–500 U/ml. Another experiment yielded similar results (not shown).

Fig. 2 shows the effect of pre-incubation of PMN with Ifn-g at a concentration of 10000 U/ml. Each cuvette contained 1.7 × 10^6 PMN; the ratio of PC:PMN was 40:1. CL was stimu-
Fig. 1. Effect of GM-CSF. Peak CL outputs from PMN incubated with varying concentrations of GM-CSF. Each column represents the mean of 3 determinations using separate aliquots of PMN. The vertical bars represent one standard deviation.

Fig. 2. Effect of interferon gamma. Peak CL outputs from PMN incubated with Ifn-g for 1 h or 2 h. Each column represents the mean of 5 determinations. The vertical bars represent one standard deviation.

Fig. 3. Effect of interferon gamma. Peak CL outputs from PMN incubated with varying concentrations of Ifn-g. Each column represents a single determination.

5. DISCUSSION

The normal immune defences against *P. carinii* infection are not well defined. Isolated congenital defects in humoral or cell-mediated immunity both predispose to PCP [3]. AIDS patients, a group particularly prone to develop PCP, lack T\(^{H}\) lymphocytes. Their monocytes exhibit decreased respiratory burst activity [4], and antibody-mediated macrophage phagocytosis is defective [5]. Histological examination demonstrates a macrophage infiltrate with few PMN in PC infected lung [6]. PMN may have roles in the acute phase of infection and in limiting the spread of PC outside the lung. Interestingly, an increase in PMNs in alveolar washings from AIDS patients with PCP correlates with a worse prognosis [11].

Macrophages are able to phagocytose PC in the absence of opsonins; attachment is mediated by the macrophage mannose receptor [12]. Opsonic antibody and complement increase phagocytosis of PC by both PMNs and macrophages [7]. Respiratory-burst activity, measured by CL, is also stimulated in both cell types if PC are preopsonised with antibody and complement [7]. CL reflects the generation of reactive oxygen moieties by phagocytes [8]. Alveolar macrophages secrete products which kill PC, but only when activated by Ifn-g; killing is abolished by the addition of catalase plus superoxide dismutase [13].

Loss of T\(^{H}\) lymphocytes in AIDS may result in a lack of various cytokines which stimulate phagocyte activity. We demonstrated in this paper the increase in CL in PMN, pretreated with lated by Ifn-g. The effect was more marked after a 2-h pre-incubation than 1 h.

Fig. 3 demonstrates the effect of preincubation of PMN with different concentrations of Ifn-g for 2 h. Each cuvette contained 2.1 \times 10^6 PMN; the ratio of PC:PMN was 37:1. There was a dose-dependent stimulation of CL in the range 5–5000 U/ml Ifn-g. These data were obtained with recombinant Ifn-g. An experiment using Ifn-g prepared from stimulated human T cells (SeraLab) yielded similar results (not shown).
GM-CSF or Ifn-g, when challenged with opsonised PC. The increase in cases of disseminated PC infection noted since the advent of AIDS [14] may be associated with defects in PMN function secondary to loss of antibody in this group [9] and/or paucity of cytokines. Also, many AIDS patients are treated with the antiretroviral drug zidovudine, which often causes neutropaenia [15]. Macrophage function may be similarly compromised in the lung. There may be a place for the therapeutic use of GM-CSF and Ifn-g in patients with PCP. However, the possibility of tissue damage caused by toxic oxygen metabolites released by phagocytes [16], perhaps by PMN in particular, must be borne in mind.

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REFERENCES