Histone preopsonisation increases the respiratory burst response of phagocytes to *Pneumocystis carinii*

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1. SUMMARY

Preincubation of *Pneumocystis carinii* with histone caused an increase in polymorphonuclear leucocyte and macrophage chemiluminescence when parasites were mixed with phagocytes compared with that obtained when unopsonised parasites were used. Toxic oxygen moieties released during the respiratory burst may cause tissue damage.

2. INTRODUCTION

*Pneumocystis carinii* is an organism of uncertain taxonomic status, probably most closely related to the fungi [1]. It causes a severe pneumonitis in patients with a variety of immunosuppressive diseases: it is the commonest secondary infection in the acquired immunodeficiency syndrome (AIDS) [2]. Histological studies show macrophages infiltrate the infected lung; polymorphonuclear leukocytes are sparse [3]. AIDS patients with pneumocystis pneumonia have a worse prognosis when the proportion of polymorphonuclear leucocytes (polymorphs) in their alveolar washings increases [4].

Histones are proteins associated with nuclear DNA in eukaryotic cells. They are released when tissue damage occurs. Histones can opsonise bacteria and yeasts causing increased phagocytosis and respiratory burst activity [5]. *P. carinii* elicits chemiluminescence, which is a consequence of the generation of reactive oxygen metabolites during the respiratory burst, in human polymorphs and rat macrophages [6]. Chemiluminescence is increased considerably if *P. carinii* organisms are preopsonised with specific antibody and complement [6]. We report here the effect of histone on this interaction.
3. MATERIALS AND METHODS

3.1. Parasites

200-g female Sprague Dawley rats (Harlan OLAC) were immunosuppressed by adding betamethasone sodium phosphate (Glaxo) to their drinking water. Tetracycline was also added to prevent bacterial infections. After 8–12 weeks animals were given broad spectrum antibiotics (amoxycillin–clavulanate, gentamicin, vancomycin and ciprofloxacin) to suppress bacterial overgrowth and killed the following day. *Pneumocystis carinii* was separated from lung homogenates by unit gravity sedimentation [7] and stored in 10% dimethyl sulphoxide at −70 °C until the day of use. This protocol routinely yielded a parasite:host cell ratio of 10^4:1 and bacterial contamination of less than 1 colony forming unit per 10^5 pneumocystis.

3.2. Phagocytic cells

Polymorphonuclear leukocytes were separated from venous blood of healthy human volunteers. 20 ml heparinised blood was mixed with 10 ml Dextran 110 injection BP (Fisons) and aggregated red blood cells were allowed to sediment for 45 min. The plasma layer was aspirated, then cells were harvested by centrifugation. Residual red blood cells were lysed by incubation in Boyle's solution (140 mM Tris, 17 mM ammonium chloride, pH 7.65) at 37 °C for 20 min. Leucocytes (in various preparations, not less than 70% polymorphs, not more than 10% monocytes, the residuum lymphocytes) were washed twice in Hanks' buffered salt solution with Hepes 20 mM pH 7.3 (HH) and then resuspended in the same medium. Foetal calf serum was added to a final concentration of 1% and the cells were incubated at 37 °C on a roller for 1 h before use. Macrophages were elicited in the peritoneal cavity of rats by the injection of 20 ml thioglycollate broth. 5 days later the peritoneal cavity of each killed animal was washed out with aliquots of HH supplemented with 0.1% gelatin (w/v; Difco) (HHg). Cells were layered onto 10 ml Lymphoprep (Nyegaard, Oslo) and centrifuged at 700 × g for 30 min. The layer above the density medium, consisting predominantly of macrophages with a few lymphocytes, was aspirated, washed twice in HHg, then resuspended in HHg with 1% foetal calf serum and incubated at 37 °C on a roller for 1 h.

3.3. Chemiluminescence

*P. carinii* organisms were thawed at 37 °C, washed twice in phosphate buffered saline, then resuspended in HH. Portions were incubated with lysine-rich histone (Sigma; ref. H5505) at concentrations indicated below for 20 min at 37 °C. The parasites were then washed twice in HH and resuspended in HH/HHg. 0.5 ml phagocytes was mixed with 0.9 ml 0.02 mM luminol (5-amino-2,3-dihydro-1,4-phthalalizinedione) in HH (polymorph experiments) or 0.2 mM lucigenin (bis-N-methylacridinium nitrate) in HHg (macrophage experiments). Baseline light outputs were determined using an LKB 1250 luminometer (Biorbit, Turku, Finland). 0.2-ml additions were made (see below) to each cuvette and serial luminometer readings taken over 30 min.

4. RESULTS

Fig. 1 shows a typical experiment with a polymorph-rich preparation. Each cuvette contained 2.3 × 10^6 leucocytes (77% polymorphs, 16% lym-
phocytes, 7% monocytes); ratio of pneumocystis:leucocytes = 29:1. Preincubation of pneumocystis with histone greatly increased the luminol-amplified chemiluminescence elicited from the leucocytes; the effect was dependent on the concentration of histone. Histone alone produced a much smaller stimulation of chemiluminescence. Two other experiments produced similar results. Lucigenin-amplified macrophage chemiluminescence was also stimulated by histone pretreatment of *P. carinii* in a concentration-dependent fashion (results not shown).

5. DISCUSSION

A consequence of the ingestion of particles such as bacteria, parasites or fungi by phagocytic cells is stimulation of the respiratory burst, in which various active oxygen species including hydrogen peroxide and superoxide ions are generated. These substances are thought to contribute to the killing of infectious agents, but may also cause tissue damage as release outside the phagocyte membrane occurs [8].

Light emission (chemiluminescence) is a result of respiratory burst activity and thus provides a measure of the interaction between phagocytes and infectious agents. Chemiluminescence can be enhanced by the addition of amplifiers such as luminol [9] and lucigenin [10]. Monocytes and macrophages generally produce very low signals when luminol is used as the amplifier. This was found when *P. carinii* was mixed with rat macrophages [6] and luminol amplified chemiluminescence thus reflects polymorph respiratory burst activity in leucocyte preparations derived from circulating blood.

Respiratory burst activity is activated by antibody and complement-coated particles; a wide range of soluble agents can also perform the same task [5]. Such agents include histones and other cationic polyelectrolytes. Like antibody, histone appears to perform optimally when attached to particles including various microorganisms such as streptococci, enterobacteria and yeasts [5]. Phagocytosis and respiratory burst activity of both polymorphs and macrophages are stimulated when specific antibody is added to pneumocystis [6]. Loss of anti-pneumocystis antibody coincided with the onset of *P. carinii* pneumonia in a group of AIDS patients; recovery of titres followed successful treatment [11]. Histological examination of lung sections from patients with pneumocystis pneumonia shows a macrophage infiltrate with few polymorphs [3]; an increase in proportion of polymorphs in bronchoalveolar washings from AIDS patients correlates with a worse prognosis [4]. Macrophages can phagocytose *P. carinii* in the absence of antibody or any other opsonin; attachment is mediated by the macrophage mannos receptor [12]. It may be that non-opsonin-mediated and antibody-targeted phagocytosis of pneumocystis by macrophages constitutes an important part of the normal protective immune response to the parasite. Release of histone by tissue damage and/or rapid parasite destruction may initiate a cycle of harmful events including an increased polymorph infiltrate followed by the release of reactive oxygen species by phagocytes, leading to further host cell destruction. Polymorphs stimulated by a wide range of opsonised particles, including pneumocystis [6], produce far higher chemiluminescence signals than macrophages. Thus polymorphs appear to produce greater amounts of reactive oxygen species than macrophages and may have a particularly prominent role in this destructive cycle.

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REFERENCES