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Serial indium-111-labelled IgG biodistribution in rat *Pneumocystis carinii* pneumonia: a tool to monitor the course and severity of the infection

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Abstract. To study the effect of new therapeutic strategies, we developed an animal model to monitor the course and severity of experimental *Pneumocystis carinii* pneumonia (PCP) in rats. *P. carinii* density scores in Giemsa-stained impression smears were used to follow *P. carinii* load. Indium-111 labelled IgG scintigraphy and biodistribution, histology of paraffin-embedded tissue sections, lung/body weight (L/B wt) ratio and cell count and differentiation of broncho-alveolar lavage (BAL) fluid were used as parameters of host inflammatory response. Statistically significant differences in L/B wt ratio, number of neutrophils in BAL fluid, *P. carinii* density score, histological extent of inflammation and ^{111}In-IgG accumulation in the lung were seen between the rats sacrificed at various time points. ^{111}In-IgG accumulation in the lung correlated well with L/B wt ratio and *P. carinii* density score and correlated moderately with number of neutrophils in BAL fluid and with the histological extent of inflammation.

Key words: Pneumocystis carinii pneumonia – Indium-111 – Biodistribution – IgG scan

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Introduction

Pneumocystis carinii pneumonia (PCP) is an important infection, causing morbidity and mortality in immunocompromised patients, especially AIDS patients [1]. Adverse reactions to effective drugs such as trimethoprim-sulfamethoxazole, clindamycin, pentamidine and trimetrexate have prompted a search for alternative therapeutic strategies [2].

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To study the effect of new therapeutic strategies, we developed an animal model to monitor the course and severity of experimental PCP in rats. The outcome of infection is determined by the balance between the offensive power of microbial pathogens and the defences of the host. Sometimes the host succumbs because of an overwhelming invasion of micro-organisms, while at other times death is due to an overwhelming inflammatory response. To evaluate therapeutic strategies, it is therefore important to monitor the growth of the micro-organisms as well as the magnitude of the host response. *P. carinii* density scores in Giemsa-stained impression smears are a good method to follow *P. carinii* load, but do not provide information on the inflammatory response of the host [3]. Histology of paraffin-embedded tissue sections gives a good impression of the extent of inflammation, but is time consuming and difficult to quantify [4, 5].

Indium-111 labelled IgG scintigraphy (IgG scan) and biodistribution are effective methods to identify local inflammation in PCP [6, 7]. These methods detect disease activity early in the course of the infection and are easy to quantify. Here we describe a model to monitor the course and severity of experimental PCP in rats, using ^{111}In-IgG biodistribution.

Materials and methods

Animal model. Four- to six-week-old, female Sprague-Dawley rats, with body weights between 148 and 175 g, were immunosuppressed with weekly subcutaneous injections of 25 mg hydrocortisone and an 8% protein-restricted diet. Amoxicillin (1 mg/ml) was added to their drinking water to prevent bacterial infections. PCP was induced by close cohabitation with *P. carinii*-infected rats. Viral co-infection was excluded by regular serological screening on common rodent viruses. Bacterial or fungal co-infection was excluded by microscopic examination of Giemsa-stained smears of the cut surface of the lung. Body weight was measured weekly.

Radiopharmaceutical and imaging protocol. Diethylene triamine penta-acetic bicyclic anhydride (bicyclic DTPA) was conjugated to HIV- and HBsAg-negative, human, non-specific, polyclonal IgG (Sandoglobulin, Sandoz AG, Neurenberg, FRG) according to the method described by Hnatowich and colleagues [8]. The conjugated solution was sterilized by membrane filtration with a 0.4 μm millipore filter and aliquots of 0.5 ml were radiolabelled with ^{111}In (indium chloride, Amersham International Ltd., Bucks., UK) via citrate transchelation. Radiochemical purity was determined by ITLC-SG chromatography (Gelman Laboratories, Ann Arbor, Mich., USA) with 0.1 *M* citrate (pH = 5) as the solvent. Labelling efficiency was always higher than 95%.

For imaging purposes, a dose of approximately 10 μg of IgG labelled with 6 MBq ^{111}In was injected intravenously via the tail vein 48 h before IgG scanning. Scintigraphic images were obtained with a Siemens Orbiter gamma-camera connected to a Scintiview image processor (Siemens Inc., Hoffmann Estates, Ill., USA). All images were collected in digital format. A quantitative analysis of intrapulmonary accumulation of ^{111}In was performed, using regions of interest in sequential IgG scans. The mean lung activity was normalized to background by dividing lung by head activity.

For biodistribution studies, lung and muscle activity was measured 48 h after injection of 10 μg IgG labelled with 1 MBq ^{111}In by counting the tissue samples in a shielded well-type gamma counter. To correct for radioactive decay and to permit calculation of the uptake of the radiopharmaceuticals in each organ as a fraction of the injected dose, aliquots of the respective dose were counted simultaneously. The activity measured in tissues was expressed as percentage of injected dose per gram. Lung to muscle ratios were calculated.

Study design. In studies such as these a relatively large number of rats is needed because in the steroid induced rat model a 15%–25% mortality due to causes other than PCP has to be anticipated. In addition the severity of PCP may vary between rats.

In experiment A, we followed 50 individual rats longitudinally. IgG scans were obtained at 2 week intervals. The animals were followed until death, whereafter the lungs were removed and the lung weight measured. Giemsa-stained impression smears of the cut surface of the left lobe were examined microscopically to determine numbers of *P. carinii*. In experiment B, a transversal study on 32 rats, we compared lung/body weight (L/B wt) ratio, number of broncho-alveolar cells, *P. carinii* density score, inflammation score and ^{111}In-IgG uptake in the lung as parameters during development of PCP. Every 2 weeks five to eight rats were given ^{111}In-IgG intravenously. Two days later the animals were killed by bleeding and a broncho-alveolar lavage (BAL) was performed [9]. Total lung weight before BAL and weights of the separate lung lobes after BAL were measured. The uptake of ^{111}In-IgG was measured in the right upper lung lobe and in a normal calf muscle sample for reference. *P. carinii* density was assessed in Giemsa- and silver-stained impression smears, extent of inflammation in paraffin-embedded tissue sections and cell count and differentiation in cytocentrifuged BAL fluid samples. The logarithmic *P. carinii* density score was assessed according to the method of Bartlett [4]. In brief, two individuals examined the slides, scoring at least ten fields per slide according to the following scheme: in one 1000× field >100 cysts = 5, 11–100 = 4, 1–10 = 3; in 10 1000× fields 2–9 = 2 and 1 cyst = 1; 0 cysts in 50 fields = 0.

Extent of inflammation in the paraffin-embedded lung sections was assessed by two examiners and scoring was as follows: 0 = normal lung histology, 1 = mild alveolar and interstitial cellular infiltrate with less than 10% of the alveoli involved, 2 = moderate interstitial and alveolar cellular infiltrate, with between 10% and 75% of the alveoli involved and 3 = severe interstitial and alveolar cellular infiltrate with more than 75% of the alveoli involved.

Statistics. The mean lung-to-head ratios at 0, 2, 4 and 6 weeks after the start of the PCP-inducing diet and corticosteroids were compared using Student's *t* test. L/b wt ratios, number of macrophages and neutrophils in BAL fluid, *P. carinii* density scores, histology inflammation scores and ^{111}In uptake were evaluated as parameters of PCP severity using a one-way analysis of variance (ANOVA). Linear regression coefficients were calculated.

The study was approved by the University Committee on Animal Experiments.

Results

In experiment A, eight rats (16%) died in the first 6 weeks or before an ^{111}In-IgG scan could be performed, while 84% of the animals died with severe PCP, 6–11 (median 8) weeks after the start of the experiment. From 2 weeks onward a steady increase in accumulation of radioactivity in the lungs was seen. The accumulation of radioactivity in the head remained the same during the course of PCP. Figure 1 shows a typical example of serial scans and lung-to-head ratios in one rat. Although at visual interpretation, pulmonary uptake seemed to increase at the various time points, mean lung-to-head ratios at the various time points (0, 2, 4 and 6 weeks) were not statistically different.

Results of experiment B are presented in Fig. 2. Nine animals died before termination of the experiment or were excluded because of bacterial or fungal contamination. In the remaining 23 animals, statistically significant differences in L/B wt ratio, number of neutrophils in BAL fluid, *P. carinii* density score, histological extent of inflammation and ^{111}In-IgG accumulation in the lung were seen between the rats sacrificed at various time points. The mean ^{111}In-IgG accumulation in the muscles was not different at the various time points. ^{111}In-IgG accumulation in the lung correlated well with L/B wt ratio ($r = 0.8$, $P<0.0001$) and *P. carinii* density score ($r = 0.8$,

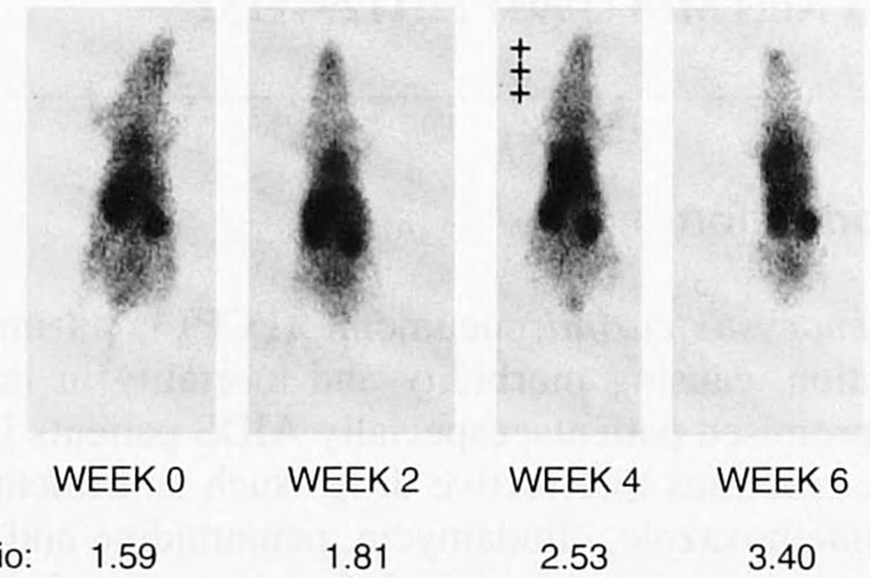

Fig. 1. Serial ^{111}Indium-IgG scans and lung-to-head ratios in *P. carinii* pneumonia in rats. N.B. Lung-to-head ratio = activity of chosen region of interest in the lung divided by activity of chosen region of interest in the head

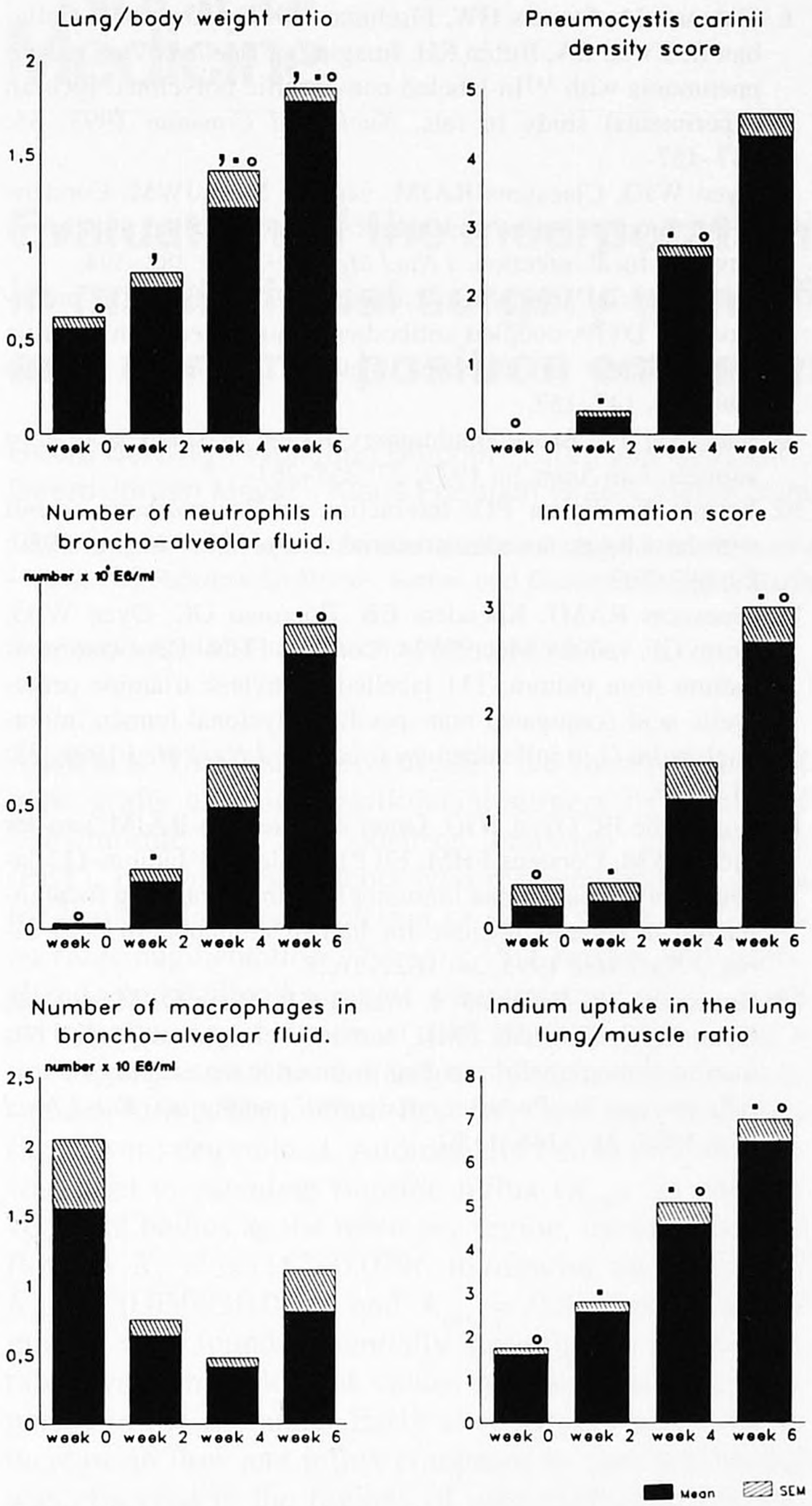

Fig. 2. Parameters of inflammation in the course of *P. carinii* pneumonia in rats. The appearance of similar signs (○, ■ and ,) above the columns denotes significant differences ($P<0.05$) at the time points in question (ANOVA)

$P<0.0001$) and correlated moderately with number of neutrophils in BAL fluid ($r = 0.6$, $P = 0.008$) and with extent of inflammation ($r = 0.6$, $P = 0.0026$). It did not correlate with number of macrophages in BAL fluid ($r = -0.38$, $P = 0.149$).

Discussion

This study shows that IgG scans and ^{111}In biodistribution are sensitive and quantitative methods to assess the severity of PCP. An early event in PCP is the alveolar-capillary leak, which follows attachment of *P. carinii* to the type 1 pneumocyte and the subsequent decrease in surface glycocalyx [10]. As shown recently, ^{111}In-IgG scintigraphy visualizes this leak. The mechanism by which ^{111}In-IgG accumulates in focal sites of infection and inflammation was recently elucidated by Claessen et al. [11]. It was shown that ^{111}In-IgG enters the inflammatory site via an increased vascular permeability. In the inflammatory field, the indium appears to be split off from the IgG and remains at that site. The IgG has been shown to leave the inflammatory site. Our data corroborate the findings by Fishman et al. in rat PCP and by Buscombe et al. in human PCP, that serial IgG scans are useful to monitor the course of PCP [6, 12, 13]. The present study gives a more precise quantification of the inflammatory parameters and demonstrates that the various parameters correlate with one another. Quantitative analysis of the inflammation can be done by comparing the ratio of lung activity with that of another (non involved) part of the body and was shown to be a reliable quantitative tool in human respiratory infections [10]. In rat PCP, however, this analysis in sequential scans was hampered by the difficulty of drawing regions due to an interfering blood pool, i.e. heart activity. The tiny lung area of the rat precluded reliable assessment and most probably caused the inability to show statistically significant increases in lung activity. However, ^{111}In biodistribution proved to be a good quantitative tool to monitor the magnitude of the alveolar-capillary leak. This parameter significantly increased at each 2-week interval and correlated well with various other parameters of severity of the infection. Lung/body weight ratio and ^{111}In uptake both mirrored the magnitude of alveolar-capillary leakage and correlated well with one another.

We were able to confirm earlier reports that the number of neutrophils increases during the course of a PCP and found that it correlates well with severity of infection.

In PCP, *P. carinii* density score in Giemsa-stained impression smears has been shown to be a reliable and reproducible indicator of *P. carinii* load [2]. In the present study, *P. carinii* density score increased gradually during infection and the score was significantly different at the time points chosen (0, 2, 4 and 6 weeks).

Histology of paraffin-embedded tissue sections gives a good impression of the extent of inflammation (the host response), but it is time consuming and difficult to quantify. The histological scoring system showed, in our hands, considerable intra- and inter-individual variation, especially early in the infection (data not shown). As the infection is patchy in that period, sampling errors are probably partly responsible. Difficulties in quantification of the inflammation and the work and time needed to prepare and evaluate the tissue sections make this method less suitable for monitoring the magnitude of host response in PCP.

In conclusion, we showed that the use of combined data on lung/body weight ratio, number of neutrophils in BAL fluid, *P. carinii* density score in Giemsa-stained impression smears and ^{111}In-IgG biodistribution in rat

PCP is a good way to monitor the course and severity of PCP. Future studies will have to show whether this method is useful for the evaluation of therapeutic strategies in PCP.

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