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Contribution of phagocytic cells and bacteria to the accumulation of technetium-99m labelled polyclonal human immunoglobulin at sites of inflammation

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Abstract. The purpose of this study was to assess the contribution of phagocytic cells and bacteria to the accumulation of technetium-99m labelled polyclonal human immunoglobulin (HIG) at sites of inflammation. Mice were intraperitoneally injected with *Staphylococcus aureus* (SA animals), with heat-inactivated newborn calf serum (NBCS, to mimic a non-bacterial inflammation) or with physiological saline (controls); 1 h thereafter they received HIG. At various intervals after the administration of HIG the mice were killed, and the percentages of radioactivity in the peritoneal effluent and attached to the cellular and bacterial fraction thereof were established. Furthermore, the total number of cells and that of bacteria in the fluid were quantitated. The percentage of activity in the effluent in the SA animals was ($P<0.02$) higher than those in the NBCS-injected animals and controls from 4 h onwards. In all groups of mice this percentage was highest at 4 h and decreased ($P<0.01$) afterwards. The percentage of cell-bound activity and the total number of cells remained fairly constant or increased with time in the SA animals ($P<0.01$). The bacteria-bound activity remained rather constant throughout the experiment and ranged between 4% and 6%. In the SA-infected animals the percentage of cell-bound activity was correlated with the total number of cells (macrophages but especially neutrophils) but even more strongly with the number of cell-associated bacteria. In the NBCS-injected animals a correlation was demonstrated between the cell-bound activity and the total number of cells (only neutrophils). It is concluded that in both experimental inflammations, phagocytic cells, and especially neutrophils, contributed significantly to the accumulation of label at the site of inflammation. Their impact on this localization is augmented by the phagocytosis of bacteria.

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Introduction

Polyclonal human immunoglobulin (Ig), labelled with either indium-111 or technetium-99m, is a well-established radiopharmaceutical for the detection of various inflammatory areas, both in experimental studies and in the clinical situation [1–6]. Labelled Ig has clear advantages over other radiotracers [7, 8], such as (a) gallium-67 citrate [9]: low radiation burden; (b) labelled leucocytes [10–12]: no time-consuming preparation; (c) labelled monoclonal antibodies [13, 14]: no formation of human anti-mouse antibodies; and (d) small chemotactic peptides [15]: no weakening of the host defence.

Despite its obvious potential, the mode of action of the accumulation of IgG at sites of inflammation is not yet fully determined [8, 16]. Most likely it involves a combination of vascular permeability [17–19], binding to cells [2, 20] and binding to bacteria [21]. All authors agree on the impact of vascular leakage with regard to the accumulation of label at the inflammation site. In contrast, there is still some debate whether binding to cells either in the circulation or localized at the inflammatory area adds substantially to this accumulation of the radiopharmaceutical. The interaction between the cells and IgG is assumed to occur via Fc receptors. Labelled Fc fragments of IgG were inferior to labelled IgG in localizing inflammations [2, 5]. Furthermore, the binding of labelled IgG to bacteria does not account for the scintigraphic detection of non-bacterial inflammation sites.

To study the contribution of cells and bacteria to the accumulation of labelled immunoglobulin at sites of in-

flammation, an experimental peritoneal model in mice was used. A bacterial inflammation was mimicked by the injection of *Staphylococcus aureus* in the peritoneal cavity, while a non-bacterial inflammation was induced by the intraperitoneal administration of newborn calf serum (NBCS) [22]. Under these experimental conditions an influx of cells from the circulation into the peritoneal cavity is to be expected: macrophages and especially neutrophils in the case of a bacterial inflammation, and macrophages and (to a lesser extent) neutrophils in the case of the non-bacterial inflammation [22]. By analysing the percentage of activity attached to cells and to bacteria in both models, the contribution of cells and bacteria can be established.

Materials and methods

Mice. Specific pathogen-free, female Swiss mice weighing 20–25 g (Broekman Instituut, Someren, The Netherlands) were used throughout the study. They were housed for 1 week before the experiments were performed. Food and water were given ad libitum.

Bacteria. Staphylococcus aureus ATCC 25923 (American Type Culture Collection, Rockville, Md., USA) was used throughout the study. This strain is serum resistant. Eighteen-hour cultures of this strain were prepared in brain heart infusion broth (BHI, Oxoid, Basingstoke, UK) in a shaking waterbath at 37° C. Aliquots of 1 ml were snap frozen in liquid nitrogen and stored at −70° C in a suspension containing about 10^9 viable bacteria per ml BHI. Just before the start of each experiment, one aliquot of this suspension was rapidly thawed in a waterbath at 37° C. The exact number of bacteria used in each experiment was quantitated by plating out appropriate dilutions on diagnostic sensitivity test agar (Oxoid). After overnight incubation at 37° C the number of bacteria was counted as colony forming units (CFUs).

Preparation of the radiopharmaceutical. As radiopharmaceutical ^{99m}Tc-labelled Technescan HIG (Mallinckrodt Medical, Petten, The Netherlands [23]) was used, a lyophilized kit containing a mixture of 1 mg 2-iminothiolane modified polyclonal human immunoglobulin (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) and stannous tartrate.

Animal model. The mice in one group (n=24) were intraperitoneally injected with approximately 3.2 (±1.1)×10^7 CFUs of *Staphylococcus aureus* in a volume of 100 µl BHI. Another group of 24 mice received an intraperitoneal injection with 100 µl of heat-inactivated NBCS (Gibco Life Technologies, Breda, The Netherlands). Finally, a third group of 24 mice received an intraperitoneal injection of 100 µl of physiological saline as control. One hour after administration all animals were anaesthetized with an intraperitoneal injection of a combination of 1 mg fluanisone, 0.03 mg fentanyl citrate (Hypnorm, Janssen Pharmaceutica, Tilburg, The Netherlands) and 0.2 mg diazepam (Valium, Hoffmann-LaRoche, Mijdrecht, The Netherlands). One hundred µg of the immunoglobulin, labelled with approximately 40 MBq ^{99m}Tc, was injected intravenously into a tail vein. Since each mouse was marked they could be followed individually. At various intervals after administration of the radiopharmaceutical six animals in each group were killed by intraperitoneal injection of 12 mg sodium pentobarbital (Sanofi BV, Division Algin, Maassluis, The Netherlands). Only for a limited period shortly after the induction of the various inflammations (approximately 10 min) did the mice looked disturbed. Using this bacterial strain in a peritoneal infection in mice the bacteria were cleared from the peritoneal cavity within 5 days (unpublished results). The animals did not show signs of poor condition during the experiments. Similar observations were made in rats.

Isolation of the peritoneal cells and bacteria. After killing the mice at various intervals after administration of the radiopharmaceutical the intraperitoneal cavity was washed by intraperitoneal administration of 2 ml of Hanks' balanced salt solution (Flow Laboratories, Irvine Ayrshire, Scotland; with HEPES, pH 7.4) at 4° C. After gentle shaking of the abdomen for 1 min, the peritoneal cells were harvested and kept at 4° C. The fluid was centrifuged for 10 min at 110×g at 4° C. The supernatant, containing the non-cell-associated bacteria, was aspirated and the pellet containing the cells and cell-associated bacteria was washed with the same medium at 4° C and resuspended in 1 ml Hanks/HEPES at 4° C. The number of cells was counted in a Bürker haemocytometer. Viability was established by trypan blue (0.2%) exclusion (always>90%). To study the cellular composition, May-Grünwald Giemsa stain was applied to cytospin preparations. The cell-free supernatant was recentrifuged for 20 min at 3000×g at 4° C. The obtained pellet containing the non-cell-associated bacteria was washed with the same medium at 4° C and resuspended in 1 ml of Hanks/HEPES at 4° C.

Determination of activity at the inflammation site. The activity in the total wash fluid, in the various pellets and in the supernatants was counted in a well-type NaI crystal detector connected to a scaler-ratemeter (SR3, Nuclear Enterprises, UK) and expressed as counts per 10 s per gram weight. The activity in each of the fractions was expressed as the percentage quantitated in that fraction to that in the total wash fluid. This percentage was considered to be an index of the activity attached to the various fractions, i.e. the cells and the bacteria.

Determination of the number of bacteria. Immediately after the determination of the amount of radioactivity the cells containing the associated bacteria were lysed by vortexing them vigorously in ice-cold distilled water for 2 min. The number of bacteria in this fraction and in the non-cell-associated fraction was determined by plating out appropriate dilutions of the respective fractions on Bouillon (Oxoid) agar. The CFUs were counted after overnight incubation at 37° C.

Statistical analysis. Differences between the various models with regard to the percentage of activity found in the cavity and bound to the cells or bacteria were analysed by means of the Student's t test [24]. The effect of time on either the percentage of activity in the various fractions or the number of cells was evaluated by means of the Kendall rank test. To detect whether the percentage of cell-bound activity was related to any other variable measured in the peritoneal cavity the Pearson correlation coefficient was calculated. To evaluate the contribution of phagocytic cells and bacteria to the accumulation of label after the injection of *Staphylococcus aureus,* multiple repression analysis [24] was used. The level of significance was set at 0.05.

Results

Percentage of activity found in the peritoneal cavity

The amount of activity found in the peritoneal cavity compared to that in the total organism after the injection of either *Staphylococcus aureus* (SA; bacterial inflammation) or NBCS (non-bacterial inflammation) or physiological saline is presented in Fig. 1. This percentage never exceeded 6% and demonstrated a peak value at 4 h after the peritoneal injection of either material. In the SA-infected animals, despite the presence of bacteria, the percentage of activity decreased 4 h after administration of the radiopharmaceutical, as was encountered in the other experiments as well ($P<0.01$). However, at 4, 18 and 24 h after administration the percentage of activity in the bacterial inflammation was significantly ($P<0.02$) higher than the percentages obtained in the other experiments.

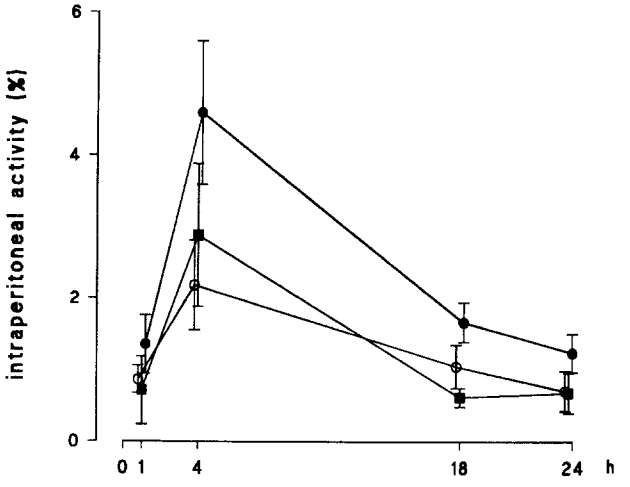

Fig. 1. The percentage of activity found in the peritoneal cavity of mice at various intervals after the administration of ^{99m}Tc-labelled immunoglobulin. The total amount of activity in the animal was taken as 100%. One hour before the administration of the radiopharmaceutical the mice received either physiological saline (○), NBCS (■) or *Staphylococcus aureus* (●). Each symbol represents the mean and standard deviation of six animals

The number and composition of peritoneal cells at various intervals after administration of radiopharmaceutical

The total number of cells throughout the experiment was highest in the SA-infected, intermediate in the NBCS-injected and lowest in the physiological saline-injected animals (Table 1). The difference was significant between the SA-infected and the animals injected with saline ($P<0.01$) at any interval after the administration of the radiopharmaceutical. In addition, significant ($P<0.001$) differences were observed between the SA-infected and NBCS-injected animals at 18 and 24 h after administration. Similar results were obtained with respect to the numbers of neutrophils. Regarding the numbers of macrophages and lymphocytes, no difference was noted between the various treatments. Only in the SA group was a time-dependent increase in the total number of cells ($P<0.001$), neutrophils ($P<0.001$) and macrophages ($P<0.01$) observed. Multiple regression analysis demonstrated that throughout our experiments the contribution of neutrophils to the total number of cells in both the SA group and the NBCS group was significantly ($P<0.01$) higher than that of macrophages, unlike in the control animals. The number of lymphocytes did not substantially influence the total number of peritoneal cells.

Table 1. The number and the composition of cells collected from the peritoneal cavity after various intervals after administration of ^{99m}Tc-labelled immunoglobulin in mice having received physiological saline (Saline), newborn calf serum (NBCS) or *Staphylococcus aureus* (*S. aureus*). Given are the means (±SD) of six observations

Cell type	Interval (h)	Total number of cells ($\times 10^5$)		
		Saline	NBCS	*S. aureus*
Total	1	9.3±6.2	16.8±7.2	26.8±7.6
	4	18.0±7.0	20.0±6.2	35.1±14.2
	18	11.6±5.6	14.8±4.7	54.0±10.9
	24	12.5±3.1	21.2±10.1	65.2±15.4
Macrophages	1	0.8±0.6	3.3±1.8	1.8±1.3
	4	1.5±1.1	3.6±2.9	2.3±1.4
	18	1.5±1.1	1.7±0.8	4.8±2.6
	24	1.8±1.1	2.5±1.7	5.0±2.1
Neutrophils	1	6.5±3.0	11.8±7.3	17.4±11.9
	4	14.6±6.1	17.0±7.1	31.0±10.0
	18	7.2±6.3	10.5±4.8	41.8±11.8
	24	9.6±1.6	15.4±7.6	56.8±13.4
Lymphocytes	1	1.5±1.5	1.8±1.8	5.0±3.7
	4	3.0±2.9	3.6±2.1	2.0±0.9
	18	1.0±0.6	2.2±1.9	5.0±3.5
	24	2.4±2.2	2.5±1.8	2.4±1.5

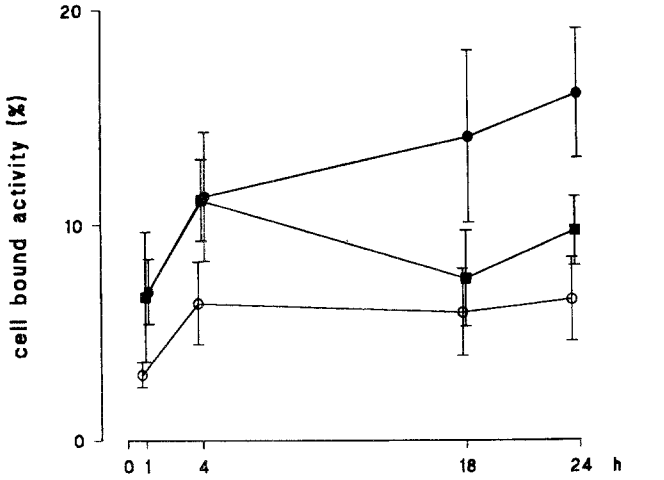

Fig. 2. The percentage of activity bound to the cellular fraction of the peritoneal effluent at various intervals after the administration of ^{99m}Tc-labelled immunoglobulin. The total amount of activity in the effluent was taken as 100%. One hour before the administration of the radiopharmaceutical the mice received either physiological saline (○), NBCS (■) or *Staphylococcus aureus* (●). Each symbol represents the mean and standard deviation of six animals

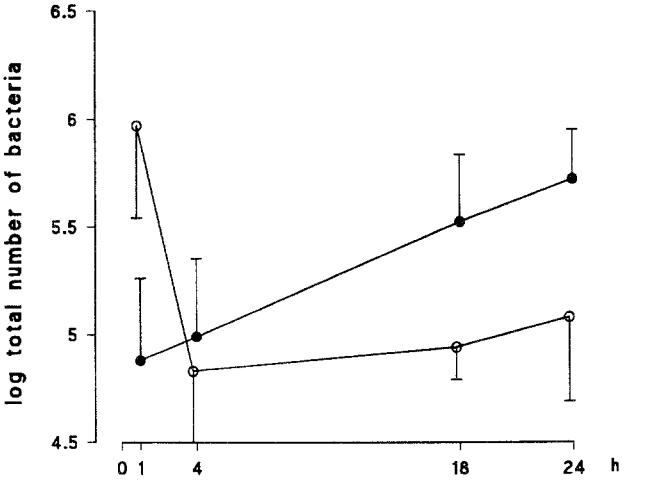

Fig. 3. The logarithmic total number of bacteria in the effluent collected from the peritoneal cavity of mice having received *Staphylococcus aureus* at various intervals after the administration of ^{99m}Tc-labelled immunoglobulin. The number was determined for both the cell-associated (●) and the non-cell-associated bacteria (○). Each symbol represents the mean and standard deviation of six animals

Percentage of activity bound to the peritoneal cells

The percentage of cell-bound activity differed between the various administrations (Fig. 2). This percentage was significantly ($P<0.05$) lower for the control animals than for the other animals throughout the experiment. The percentage of label was significantly ($P<0.05$) higher at 18 and 24 h after administration in the SA-inoculated than in the NBCS-injected animals. Only in the case of bacterial inflammation was a time-dependent ($P<0.01$) increase in the percentage of cell-bound activity noted. In the other two groups it remained at an approximately constant level throughout the experiment.

The number of bacteria in the peritoneal cavity

Using differential centrifugation techniques, distinction was made between the cell-associated bacteria, either phagocytosed or adhered, and the free bacteria in the peritoneal cavity. The respective numbers throughout the experiment are depicted in Fig. 3. At 1 h after the administration of the labelled IgG (corresponding to 19 h after infection) the number of the free bacteria was about tenfold that of the cell-associated bacteria. From 4 h onwards the latter exceeded the former. In contrast to the number of free bacteria, the cell-associated bacteria demonstrated a time-dependent ($P<0.01$) outgrowth, starting from about 9.0×10^4 at 1 h to about 7.5×10^5 CFUs at 24 h. This implies that the contribution of the cells which phagocytosed bacteria increased with time. The number of free bacteria rapidly declined between 1 and 4 h. Until 24 h this number remained approximately the same. Thus, the infection was not cleared within 24 h after administration of the labelled compound.

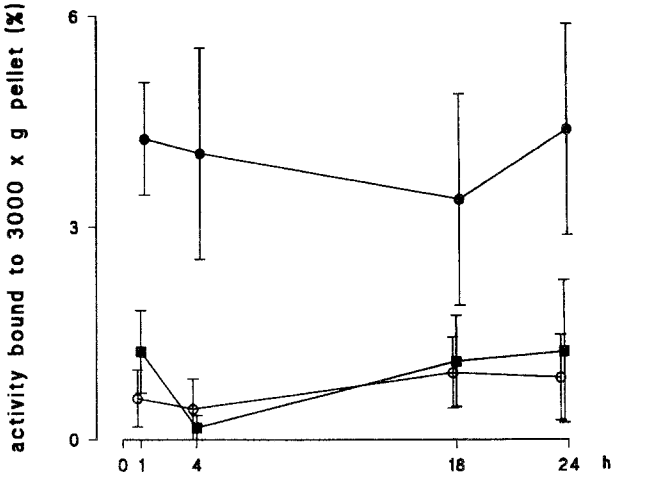

Fig. 4. The percentage of activity bound to the 3000×*g* pellet of the cell-free peritoneal effluent at various intervals after the administration of ^{99m}Tc-labelled immunoglobulin. The total amount of acivity in the effluent was taken as 100%. One hour before the administration of the radiopharmaceutical the mice received either physiological saline (○), NBCS (■) or *Staphylococcus aureus* (●). Each symbol represents the mean and standard deviation of six animals

Percentage of activity bound to bacteria

As demonstrated in Fig. 4, the percentage of activity in the 3000×*g* pellet of the cell-free fraction of the peritoneal effluent was low. In the SA-infected animals this pellet represented the non-cell-associated bacteria and ranged between 2% and 6% of the total amount of activity in the peritoneal cavity. Between the various intervals there was no substantial change in this percentage. In the other groups, without a bacterial fraction, the percentage

found in the pellet after the second centrifugation step was less than 2%. This could be attributed to a small remaining cell population or aggregated labelled immunoglobulin molecules.

Correlations between the cell-bound activity and the various variables in the peritoneal cavity

Only in the SA-infected animals from 4 h onwards was an inverse ($P<0.001$) correlation demonstrated between the percentage of activity in the peritoneal cavity and that found in the cell-bound fraction. By adjusting the percentage of activity to the same level from 4 h onwards, the cell-bound activity increased significantly ($P<0.01$) in all groups, especially in the SA-infected animals ($P<0.001$). This suggests an increase in the contribution of cells present at the site of inflammation to the accumulation of label in the peritoneal cavity, especially in the case of the SA-infected animals.

In all groups of mice a positive ($P<0.01$) correlation between the cell-bound activity and the total number of cells collected from the peritoneal cavity was demonstrated. In the case of both the bacterial and the non-bacterial inflammation it was found that a positive ($P<0.001$) correlation existed between the cell-bound activity and the number of neutrophils. No such correlation was established between the cell-bound activity and the number of macrophages. Concerning the bacteria-bound activity, a positive ($P<0.01$) correlation was detected with the number of neutrophils. Furthermore, a positive correlation between the cell-bound activity and both the total number of cells ($P<0.02$) and the number of cell-associated bacteria ($P<0.01$) was found. Analysis of variance resulted in an F value of 9.97 ($P=0.002$). No correlation was observed between the percentage of cell-bound activity and the number of free bacteria.

Discussion

The main conclusion from this experimental study is that both phagocytic cells and cell-associated bacteria contribute significantly to the accumulation of ^{99m}Tc-labelled polyclonal human immunoglobulin at the site of a peritoneal infection with *S. aureus*. Furthermore, it was noted that this cell-bound activity was correlated with the total number of cells, especially the number of neutrophils. In this respect it was important that cell-associated bacteria also showed a correlation with the cell-bound activity, suggesting that phagocytosis of bacteria enhances this cell-mediated activity. In contrast, a contribution of non-cell-associated bacteria to the accumulation was not established. In an inflammation induced by NBCS, the phagocytic cells, and especially neutrophils, contribute significantly to the accumulation of ^{99m}Tc-labelled immunoglobulin. Saptogino et al. [20] demonstrated in vitro binding of ^{99m}Tc-labelled immunoglobulin to blood monocytes and to a much lesser extent to neutrophils. However, in that study a relatively high concentration of immunoglobulin was employed, using unstimulated blood cells. It is clear that under these circumstances the neutrophils were not essential since in these experiments no inflammation was involved. Morrel et al. [17] did not find any important binding of labelled IgG to cells at the site of an infection with *Pseudomonas aeruginosa* by means of an autoradiographic method. It must be emphasized that in the latter study a histological method was applied with Gram-negative micro-organisms in a deep thigh inflammation.

At a site of inflammation due to the presence of antigens the phagocytic cells are stimulated and thereby possess, among other things, a higher number of Fc receptors on their cell surface than in the unstimulated situation [25]. However, various studies have suggested that Fc receptor binding does not play a major role in the localization of labelled IgG at sites of bacterial inflammation [5, 26]. An option for the cell-associated activity is that these cells phagocytose immune complexes, as Kupffer cells are known to do [27, 28], or use other receptors for the binding to labelled immunoglobulin.

The percentage of activity associated to bacteria was low in the present study, never exceeding 6%. In a thigh inflammation with the same micro-organism [21] it was found that a specific binding to the protein A content of *S. aureus* influenced the accumulation of labelled IgG. Furthermore, the degree of accumulation of the radioactive tracer was also associated with the number of bacteria [5]. In the present study a relatively low inoculum of a strain of *S. aureus* was used, containing a moderate percentage of protein A on the cell surface. It is likely that these factors influence the binding avidity of the labelled immunoglobulin.

The strong correlation between the cell-bound activity and the number of cell-associated bacteria exceeded the correlation between the cell-bound activity and the number of cells. Moreover, the contribution of neutrophils to the cell-bound activity was higher than that of macrophages. These findings suggest that the cells phagocytosed bacteria bound to labelled IgG or at least that they came into contact with these bacteria, this being the initial step in phagocytosis. This might also explain the significant difference in cell-bound activity between the SA-infected animals and the animals that received NBCS. In the latter the contribution of the bacteria is lacking. The same holds for the percentage of activity in the peritoneal cavity. In a bacterial inflammation neutrophils are attracted to the inflammation site and these cells are more suited for the phagocytosis and killing of bacteria than are macrophages [29, 30]. The same mechanism might account for the importance of neutrophils in the NBCS non-bacterial inflammation, as the stimulation of macrophages may result in a short-term influx of circulating neutrophils. These considerations might explain why the target to non-target ratios in non-bacterial inflammation are substantially lower than those detected in bacterial inflammation [3, 31, 32].

In a previous experimental study in mice [5] an increase in the number of bacteria in the inoculum was accompanied by an increase in the target to non-target ratio in infected thigh tissue. This observation could be explained not only by a higher number of bacteria but also by a higher number of cells, especially neutrophils, at the inflammation site. As was demonstrated in an experimental lung infection [33], the number of neutrophils was associated with the number of bacteria at the site of the inflammation. Furthermore, these neutrophils appeared to have phagocytosed a substantial number of bacteria.

To ensure that the activity detected in either the cell fraction or the bacteria fraction was specific, a control experiment (with determinations at 4 and 24 h after administration of the radiopharmaceutical) was performed using ^{99m}Tc-labelled human serum albumin (HSA). Monomeric HSA was used since di- and polymeric HSA, the latter mimicking the molecular weight of immunoglobulin more closely, yielded lower target to non-target ratios in an experimental model [5]. If the results obtained with the monomeric fraction of HSA, which is smaller than immunoglobulin, were to be significantly lower than those obtained with HIG, the difference between HIG and di- and polymeric HSA would be even greater. At both intervals after the administration of labelled monomeric HSA the percentage of activity in the cell-bound fraction was lower than 3%. This was significantly lower than was observed for ^{99m}Tc-labelled immunoglobulin at the same intervals.

It is concluded that several inflammatory mechanisms contribute to the accumulation of ^{99m}Tc-labelled IgG at sites of inflammation:

1. Vascular permeability allowing the influx of labelled IgG
2. A specific binding of labelled IgG to the cells present at the site of inflammation, and especially to neutrophils
3. A specific binding of labelled IgG to the bacteria present at the site of inflammation, this binding being dependent on the number and the strain of bacteria involved (Gram-positive versus Gram-negative)
4. The phagocytosis of bacteria by cells allowing the adherence or entrance of labelled IgG to these cells

It is likely that all these mechanisms contribute to the accumulation of radiolabelled polyclonal human immunoglobulin at the site of inflammation.

References

1. Rubin RH, Fischman AJ, Needleman M, Wilkinson R, Callahan RJ, Khaw BA, Hansen WP, Kramer PB, Strauss HW. Radiolabelled, nonspecific, polyclonal human immunoglobulin in the detection of focal inflammation by scintigraphy: comparison with gallium-67 citrate and technetium-99m-labelled albumin. *J Nucl Med* 1989; 30: 385–389.
2. Fischman AJ, Rubin RH, Khaw BA, Callahan RJ, Wilkinson R, Keech F, Nedelman M, Dragotakes S, Kramer PB, LaMuraglia GM. Detection of acute inflammation with ^{111}In-labelled nonspecific polyclonal IgG. *Semin Nucl Med* 1988; 18: 335–344.
3. Sciuk J, Brandau W, Vollet B, Stücker R, Erlemann R, Bartenstein P, Peters PE, Schober O. Comparison of technetium 99m polyclonal human immunoglobulin and technetium 99m monoclonal antibodies for imaging chronic osteomyelitis. *Eur J Nucl Med* 1991; 18: 401–407.
4. Buscombe JR, Lui D, Ensing G, de Jong R, Ell PJ. ^{99m}Tc-human immunoglobulin (HIG) – first results of a new agent for the localization of infection and inflammation. *Eur J Nucl Med* 1990; 16: 649–655.
5. Calame W, Feitsma HIJ, Ensing GJ, Goedemans WT, Camps JAJ, van Furth R, Pauwels EKJ. Detection of a local staphylococcal infection in mice with ^{99m}Tc-labelled polyclonal human immunoglobulin. *J Nucl Med* 1991; 32: 468–474.
6. Rubin RH, Fischman AJ, Callahan RJ, et al. In-111 labeled nonspecific immunoglobulin scanning in the detection of focal infection. *N Engl J Med* 1989; 321: 935–940.
7. Calame W, Feitsma HIJ, Pauwels EKJ. Scintigraphic detection of inflammatory disease by various radiopharmaceuticals, especially labelled immunoglobulin. *Nuklearmedizin* 1992; Suppl 28: 260–269.
8. Calame W, Welling M, Feitsma HIJ, Ensing GJ, Pauwels EKJ. Improved detection of a staphylococcal infection by monomeric and protein A-purified polyclonal human immunoglobulin. *Eur J Nucl Med* 1993; 20: 490–494.
9. Lavender JP, Lowe J, Barker JR, Burn JI, Chaudri MA. Gallium-67 citrate scanning in neoplastic and inflammatory lesions. *Br J Radiol* 1971; 44: 361–366.
10. McAfee JG, Thakur ML. Survey of radioactive agents for in vitro labelling of phagocytic leukocytes. I. Soluble agents. *J Nucl Med* 1976; 17: 480–487.
11. Danpure HJ, Osman S, Brady F. The labelling of blood cells in plasma with ^{111}In-troponolate. *Br J Radiol* 1982; 55: 247–249.
12. Peters AM, Danpure HJ, Osman S, Hawker RJ, Henderson BL, Hodgson HJ, Kelly JD, Neirinckx RD. Clinical experience with ^{99m}Tc-hexamethylpropylenamineoxime for labelling leukocytes and imaging inflammation. *Lancet* 1986; II: 946–949.
13. Locher JTH, Seybold K, Andres RY, Schubiger PA, Mach JP, Buchegger F. Imaging of inflammatory and infectious lesions after injection of radioiodinated monoclonal antigranulocyte antibodies. *Nucl Med Commun* 1986; 7: 659–670.
14. Rubin RH, Young LS, Hansen WP, Nedelman M, Wilkinson R, Nelles MJ, Callahan R, Khaw BA, Strauss HW. Specific and nonspecific imaging of localized Fisher immunotype I *Pseudomonas aeruginosa* infection with radiolabelled monoclonal antibody. *J Nucl Med* 1988; 29: 651–656.
15. Fischman AJ, Pike MC, Kroon D, Fucello AJ, Rexinger D, TenKate C, Wilkinson R, Rubin RH, Strauss HW. Imaging focal sites of bacterial infection in rats with indium-111-labelled chemotactic peptide analogs. *J Nucl Med* 1991; 32: 483–491
16. Corstens FHM, Claessens RAMJ. Imaging inflammation with human polyclonal immunoglobulin: not looked for but discovered. *Eur J Nucl Med* 1992; 19: 155–158.
17. Morrel EM, Tompkins RG, Fischman AJ, Wilkinson RA, Burke JF, Rubin RH, Strauss HW, Yarmush ML. Autoradiographic method for quantitation of radiolabelled proteins in tissues using indium-111. *J Nucl Med* 1989; 30: 1538–1545.
18. Thakur ML, DeFulvio J, Park CH, et al. Technetium-99m-labelled proteins for imaging inflammatory foci. *Nucl Med Biol* 1991; 18: 605–612.

19. Thakur ML, Defulvio J, Park CH, Epstein A, McAfee J. Tc-99m HSA for imaging inflammatory foci [abstract]. *Eur J Nucl Med* 1990; 16: 307.
20. Saptogino A, Becker W, Wolf F. ^{99m}Tc-labelled polyclonal human immunoglobulin for localization of inflammatory sites – early in vitro results. *Nuklearmedizin* 1990; 29: 54–58.
21. Calame W, Feitsma HIJ, Ensing GJ, Arndt JW, van Furth R, Pauwels EKJ. Binding of ^{99m}Tc-labelled polyclonal human immunoglobulin to bacteria as a mechanism for scintigraphic detection of infection. *Eur J Nucl Med* 1991;18: 396–400.
22. Van Waarde D, Hulsing-Hesselink E, van Furth R. A serum factor inducing monocytosis during an acute inflammatory reaction caused by newborn calf serum. *Cell Tissue Kinet* 1976; 9: 51–63.
23. Goedemans WT, Panek KJ. A new, simple method for labeling of proteins with ^{99m}Tc [abstract]. *J Nucl Med Allied Sci* 1989; 33: 286.
24. Snedecor GW, Cochran WG. *Statistical methods, 7th edn.* Ames, Iowa: The Iowa State University Press, 1980.
25. Roitt I. *Essential immunology, 6th edn.* Oxford: Blackwell Scientific; 1988: 154–171.
26. Fischman AJ, Fucello AJ, Pellegrino-Gensey JL, Geltofsky J, Yarmush ML, Rubin RH, Strauss HW. Effect of carbohydrate modification on the localization of human polyclonal IgG at focal sites of bacterial infection. *J Nucl Med* 1992; 33: 1378–1382.
27. Skogh T, Blomhoff R, Eskild W, Berg T. Hepatic uptake of circulating IgG immune complexes. *Immunology* 1985: 55: 585–594.
28. Rifai A, Mannik M. Clearance of circulating IgA immune complexes is mediated by a specific receptor on Kupffer cells in mice. *J Exp Med* 1984; 160: 125–137.
29. Harmsen AG. Role of alveolar macrophages in lipopolysaccharide-induced neutrophil accumulation. *Infect Immun* 1988; 56: 1858–1863.
30. Ozaki T, Maeda M, Hayashi H, Nakamura Y, Moriguchi H, Kamei T, Yasuoka S, Ogura T. Role of alveolar macrophages in the neutrophil-dependent defense system against *Pseudomonas aeruginosa* infection in the lower respiratory tract. Effect of muramyl dipeptide analog. *Am Rev RespirDis* 1989; 140: 1595–1601.
31. De Bois MHW, Arndt JW, van der Velde EA, Pauwels EKJ, Breedveld FC. Joint scintigraphy for quantification of synovitis with ^{99m}Tc-labelled human immunoglobulin G compared to late phase scintigraphy with ^{99m}Tc-labelled diphosphonate. *Br J Rheumatol* 1994; 33: 67–73.
32. Van der Lubbe PAHM, Arndt JW, Calame W, Ferreira TC, Pauwels EKJ, Breedveld FC. Measurement of synovial inflammation in rheumatoid arthritis with technetium-99m labelled human polyclonal immunoglobulin G. *Eur J Nucl Med* 1991; 18: 119–123.
33. Calame W, Douwes-Idema AE, van den Barselaar MT, van Furth R, Mattie H. Influence of cytostatic agents on the pulmonary defense in mice infected with *Klebsiella pneumoniae* and on the efficacy of ceftriaxone treatment. *J Infect* 1994: 29: 53–66.