Imaging of Inflammatory Arthritis with Technetium-99m-Labeled IgG

Ferdinand C. Breedveld, Marinus J.P.G. van Kroonenburgh, Jan A.J. Camps, Hans I.J. Feitsma, Harry M. Markusse, and Ernest K.J. Pauwels

Departments of Rheumatology and Diagnostic Radiology (Division of Nuclear Medicine), University Hospital, Leiden, The Netherlands

The accumulation of nonspecific polyclonal human immunoglobulin G (IgG) radiolabeled with ^{99m}Tc was compared to that of [^{99m}Tc]albumin and [^{99m}Tc]nanocolloid in rats with collagen induced arthritis. Serial scintigrams were acquired directly, 4 and 24 hr after injection. A clearly discernable image of the site of synovitis was seen with [^{99m}Tc]IgG as early as 4 hr postinjection. The relative intensity of the inflammatory lesion was maximal at 24 hr. Discrimination between arthritic and nonarthritic joints as well as correlations between the relative intensity of the arthritic joint and clinical indices of joint inflammation were superior with IgG compared to albumin or nanocolloid. These studies show that localization and severity of inflammatory joint disease can be detected with radiolabeled nonspecific IgG.

J Nucl Med 30:2017–2021, 1989

Radiolabeled IgG scintigraphy has been recognized as a reliable modality for localization and evaluation of pyogenic infections (*1–3*). Intravenously administered, nonspecific, polyclonal IgG accumulates at the site of inflammation and produces target to background ratios which were adequate for imaging purposes. Radiolabeled IgG showed a greater accumulation at the site of inflammation than both gallium-67 citrate and radiolabeled albumin (*2*). These attributes of IgG prompted us to evaluate the suitability of this technique for imaging inflammatory activity in autoimmune arthritis. Images of the collagen model of chronic arthritis in rats obtained with ^{99m}Technetium labeled human IgG ([^{99m}Tc]IgG) were compared with those obtained with [^{99m}Tc]human serum albumin (HSA) and ^{99m}Tc nanometer sized albumin colloid (nanocolloid).

MATERIALS AND METHODS

Rats

Collagen arthritis (CA) was induced in outbred female Spraque-Dawley rats (Broekman, Someren, The Netherlands) weighing 150–200 g, by intradermal injection on Day 0 with 0.4 mg native chick type II collagen (Genzyme, Boston, MA) solubilized in 0.1*M* acetic acid and emulsified in incomplete Freund's adjuvant. The presence and severity of hindlimb arthritis were determined clinically using the conventional arthritic index in which limbs were graded 0, 1, 2, 3, or 4 representing increasing erythema and swelling (*4–6*).

Scintigraphy

Human polyclonal IgG (Central Laboratory of the Red Cross Bloodtransfusion Service, Amsterdam, The Netherlands) was labeled as previously described. In brief, a mixture of [^{99m}Tc]pertechnetate (200 μl in saline), dimethylformamide (11 μl) and 5 N hydrochloric acid (3 μl) is heated at 140°C for 4 hr. The remaining dry residue is extracted with chloroform (100 μl). The chloroform solution, containing the ^{99m}Tc activity, is transferred to another tube and evaporated to dryness. The small residue is designated as intermediate. Then 200 μl of a solution of the IgG (10 mg/ml in saline) is added to the dry intermediate and incubated at 40°C for 60 min. Typically, labeling yields are higher than 95% (*7*). The IgG preparation is prepared for intravenous administration and contains after ^{99m}Tc-labeling <2% IgG aggregates. A kit-prepared (^{99m}Tc)-nanocolloid and [^{99m}Tc]HSA (Solco, Basel, Switzerland) was used. The labeling efficiency of the preparations was either checked by thin layer chromatography or was performed after precipitation with 20% trichloracetic acid and was found to be 90%. The rats were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories). Each animal was injected i.v. through a tail vein with 1.0 mCi of the preparation of either [^{99m}Tc]IgG; (^{99m}Tc)-nanocolloid or (^{99m}Tc)-HSA. Non-immunized littermate controls were similarly injected.

A small field of view gamma camera (Toshiba, GCA 102s) with a high resolution parallel hole collimator and connected to a MDS-A2 computer was used for whole body imaging. The energy peak was set at 140 keV with a 20% window. The

Received Mar. 23, 1989; revision accepted July 21, 1989.

For reprints contact: F.C. Breedveld, MD, University Hospital, Dept. of Rheumatology, Building 1, C2-Q, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

animals were positioned ventral side facing the collimator and serial scintigrams were carried out immediately, 4 and 24 hr postinjection. Images were acquired in a 256 × 256 matrix. Static images contained 300.000 counts. An objective score was determined from the scintigrams as described previously by De Sousa et al. (*8*). Regions of interest, i.e., right and left knees and ankles were scored. The score was defined as the ratio of the counts per pixel in the region of interest over the counts per pixel in the adjacent tissue, corrected for background. Basically, the amount of background activity was similar in all experiments. Adjacent tissue activity was measured in noninflammed tissue of the upper leg and background activity was measured outside the animal contour. There was little difference in background activity between the different experiments. The scintigrams were subjectively scored by two experienced observers and graded as normal, possibly abnormal or abnormal. The observers were not involved in the immunization or handling of the rats and were without knowledge of the objective score or the arthritic index reflecting the clinical severity of the inflammation.

Statistical analysis

Continuous variables were analyzed in terms of their group means (Student's t-test) and by linear regression.

RESULTS

Rats with CA had serial scintigrams carried out with [^{99m}Tc]IgG administered intravenously. Sites of inflammation were seen at 4 hr postinjection of the [^{99m}Tc]IgG, with increasing relative intensity of the lesion observed at 24 hr postinjection (Fig. 1 and Figure 2A). Most images of arthritic animals were considered subjectively as abnormal only 24 hr after injection of [^{99m}Tc]IgG. A total of 31 images were recorded at 24 hr postinjection from 27 immunized and four nonimmunized rats (Fig. 2B). Of these images 41 ankles were considered abnormal and 19 normal by subjective analysis. Two were graded as possibly abnormal. The mean objective score in the joints with abnormal subjective analysis was significantly higher than the mean objective score in the group with normal subjective scores (1.68 ± 0.43 vs. 0.65 ± 0.15, respectively, $p < 0.0001$).

Comparison of this scintigraphic technique with clinical indices of arthritis activity are shown in Figure 3A. Mildly affected hindlimbs (arthritic index 1) were not detected with the [^{99m}Tc]IgG imaging technique. Only one of the nine images of moderately affected ankles (arthritic index 2) was considered normal and all images of severe and very severe ankle arthritis (arthritic index resp. 3 and 4) were subjectively scored as abnormal. Linear regression analysis revealed that increasing severity of clinical arthritis highly correlated with increase of objective scores ($R = 0.33$, $p = 0.0006$). Analysis of the objective scores according to the time postimmunization (Fig. 3B) indicated that the difference between the objective scores of immunized and nonimmunized

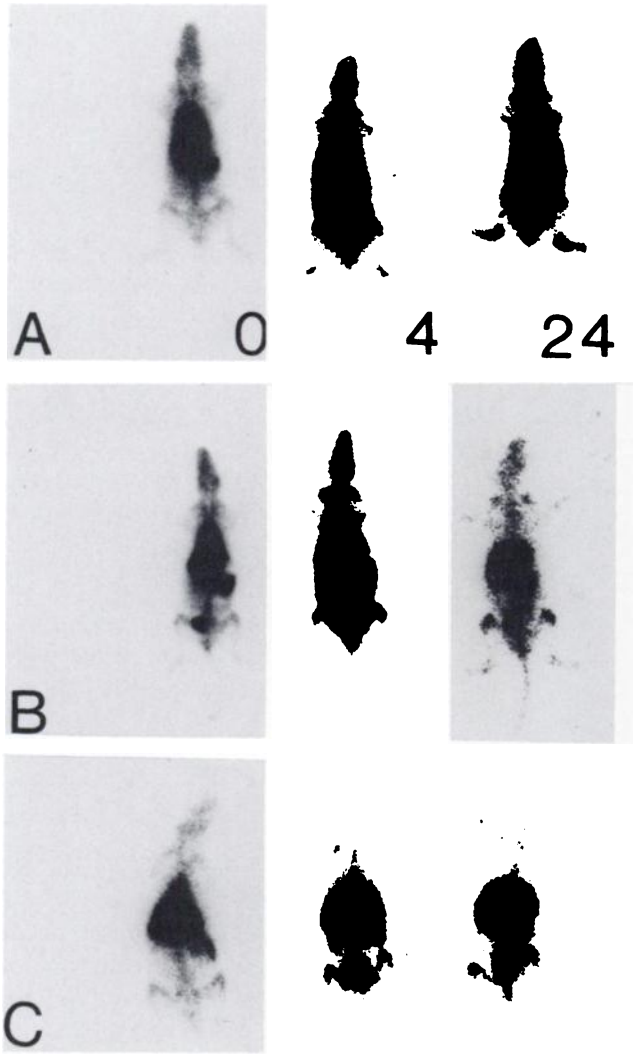

FIGURE 1
Serial scintigrams in rats with collagen arthritis who have been injected with ^{99m}Tc-labeled, nonspecific human IgG (A), ^{99m}Tc-labeled human serum albumin (B) and ^{99m}Tc-labeled nanocolloid (C). The rats were imaged at the indicated number of hours postinjection.

groups became maximal between 28 and 30 days after immunization.

The mean objective score of the knees in immunized rats was significantly higher compared to nonimmunized rats (respectively, 2.76 ± 0.81 and 1.67 ± 0.64 $p < 0.01$). Thirteen of the 52 knees from rats with clinical evidence of ankle arthritis had objective scores higher than the mean plus two s.d.s of objective scores in nonimmunized rats. This number is close to the incidence of 20% microscopically proven knee arthritis in previous studies of arthritic animals in this experimental model of chronic arthritis (*8*).

Comparison with Other Radiopharmaceuticals

Scintigraphy carried out with [^{99m}Tc]HSA produced images of the site of inflammation that were less clearly discernable compared to the IgG scintigrams (Fig. 1).

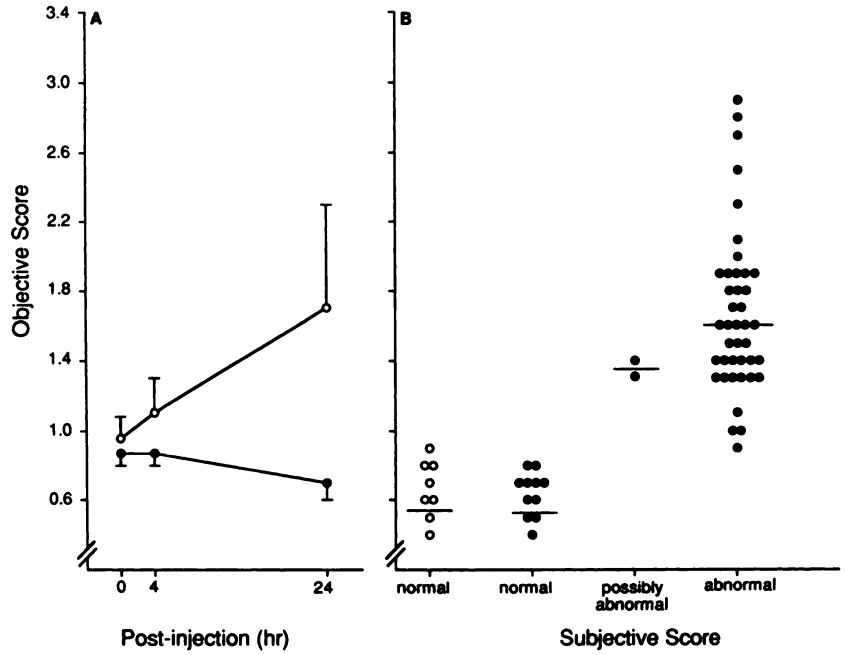

FIGURE 2
A: Graphic representation of the ratio of the numbers of counts per pixel at the site of ankle arthritis divided by the number of counts per pixel of adjacent tissue (=objective score depicted as mean ± s.d.) demonstrating changes in target-background ratio in the first 24 hr after injection of ^{99m}Tc-labeled nonspecific human IgG [^{99m}Tc]IgG in 12 arthritic rats immunized with collagen (●) or in four nonimmunized littermates (○). B: Comparison of subjective and objective scores using the [^{99m}Tc]IgG imaging technique to measure arthritic involvement in ankles of rats immunized with type II collagen (●) and nonimmunized littermates that served as control (○). Horizontal lines indicate group means.

A total of nine images from six immunized and three nonimmunized rats were evaluated. With [^{99m}Tc]HSA the objective score of all ankles at 4 hr postinjection (1.72 ± 0.63) was higher than that measured directly after injection (1.11 ± 0.37, $p < 0.01$) and 24 hr postinjection (1.50 ± 0.91, NS) (Fig. 4A). Although the mean objective score of arthritic ankles measured at 4 hr postinjection was significantly higher compared to nonarthritic ankles of nonimmunized animals (respectively, 1.99 ± 0.49 and 1.31 ± 0.53, $p < 0.05$), six of the 12 images of arthritic ankles had objective scores within the range of nonarthritic ankles. No correlation was found between objective scores of ankle inflammation and clinical indices of arthritis activity (data not shown).

Scintigraphy with [^{99m}Tc]nanocolloid produced images of ankles from arthritic rats that were less clearly discernable compared to both IgG and HSA scintigraphy. Ten images from seven immunized and three nonimmunized rats were evaluated. The objective scores measured 4 hr after injection did not differ significantly of those measured after 24 hr in animals (1.4 ± 0.43 and 1.4 ± 0.39, respectively; Fig. 4B). The mean objective score of arthritic ankles was higher than that of nonarthritic ankles but the difference was not significant (respectively, 1.5 ± 0.45 and 1.1 ± 0.36) at 4 hr postinjection). Also with nanocolloid, no clear correlation was found between objective scores of ankle inflammation and clinical indices of arthritis activity (data not shown).

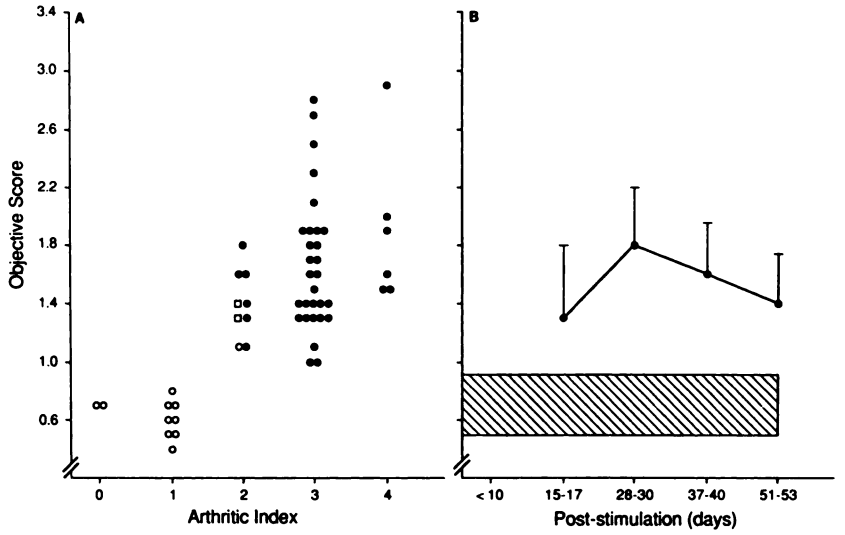

FIGURE 3
A: Depiction of objective scores versus arthritic indices for ankles of rats immunized with type II collagen. Images scored subjectively as normal are represented by open circles, as abnormal by closed circles and as possibly abnormal by squares. B: Course of collagen arthritis, depicted as the mean objective score ± s.d. for ankles of 16 immunized rats investigated at different periods after immunization. Shaded areas represent mean ± s.d. of control values for nonimmunized rats.

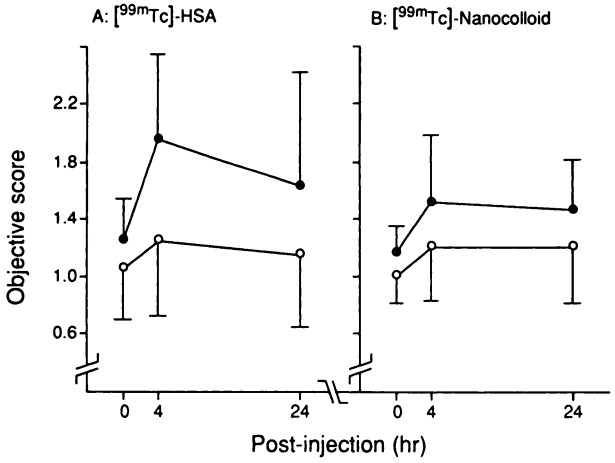

FIGURE 4
Depiction of objective scores ± s.d. for ankles of rats measured directly, 4 or 24 hr after injection. A: injection of [^{99m}Tc]HSA in six immunized (●) and three nonimmunized rats (○), B: injection of [^{99m}Tc]nanocolloid in seven immunized (●) and three nonimmunized rats (○).

DISCUSSION

Radioimaging with ^{99m}Tc-labeled human polyclonal IgG was shown to be capable of measuring the localization and severity of collagen arthritis in the rat. Recent studies by Rubin et al. (*1–3*) demonstrated that human IgG accumulates at focal sites of inflammation in both rats and human subjects with bacterial infection. It was shown that the accumulation of IgG was the result of a nonspecific process and that an intact Fc portion of IgG is necessary for concentration at the site of inflammation. Comparison with albumin scintigraphy of pyogenic infection showed that the relative concentration of radiolabeled IgG is far higher than that of radiolabeled albumin which suggests a specific trapping mechanism for IgG (*2*).

The results of the present study demonstrate that IgG accumulation also occurs in inflammation based on autoimmune mechanisms. IgG accumulation could be measured up to 24 hr after injection whereas with albumin egress from the inflammatory site equals the ingress shortly after injection. Although IgG was superior to albumin in the discrimination of arthritic from nonarthritic joints our results are in contrast with previous studies (*2*) by not showing higher objective scores of the inflammatory lesions measured with IgG than with HSA. If the capture of IgG occurs via Fc receptors, these contrasting results might be explained by the type of inflammatory cells at the site of inflammation. Affected tissue in collagen arthritis is predominantly infiltrated by lymphocytes and macrophages which may express less Fc receptors compared to the polymorphonuclear leukocytes that dominate pyogenic infections (*4*). However, the role of Fc receptors in the accumulation of IgG at sites of inflammation remains to be determined. An alternative explanation for the continued accumulation at inflammatory sites of IgG and not of albumin might be the larger molecular size of IgG. Therefore we also studied scintigraphy with [^{99m}Tc]IgG versus ^{99m}Tc-nanometer-sized albumin-based colloid. This technique was recently shown to be effective in the detection of inflammation (*9*). For the detection of collagen arthritis scintigraphy with nanocolloid was inferior to IgG scintigraphy. The poor accumulation of nanocolloid at the site of inflammation supports the suggestion that the capture of IgG at the inflammatory site occurs via the typical immunological or physichochemical properties of the IgG molecule.

The scintigraphic findings with [^{99m}Tc]IgG in rats with different duration of arthritis correlated well with pathologic findings of synovial hypervascularity, pannus formation, and inflammatory cell infiltrate seen in previous microscopic studies on the course of arthritis in this model (*6–8*). This and the correlation observed between clinical and scintigraphic assessment of ankle arthritis indicate that in this model [^{99m}Tc]IgG uptake is an accurate way of detecting and measuring arthritis. Experimentally induced autoimmune arthritis in rodents is widely used in studies on pathogenesis and therapy of chronic arthritis (*10*). Since proximal joints such as the knees cannot be evaluated clinically in these models, IgG scintigraphy can be employed to evaluate all joints involved in collagen arthritis.

In the evaluation of disease activity in rheumatoid arthritis many clinical and laboratory tests have been employed. However, the goal of objectivity in quantitation has largely remained elusive. Joint scintigraphy has the potential to provide an objective noninvasive way of measuring synovial involvement in arthritis (*11*). The suggested mechanisms of IgG scintigraphy include exudation of plasmaproteins through a leaking capillary bed at sites of inflammation and specific trapping of IgG by inflammatory cells. These mechanisms are intimately linked to the pathophysiology of inflammations.

Therefore the information obtained with IgG scintigraphy may be more specific for inflammatory activity than that obtained with previously studied radiopharmaceuticals such as technetium labeled pertechnetate or phosphates (*12*). The results of the present study serve to contribute that further evaluation of [^{99m}Tc]IgG radioimaging techniques in human arthritis deserves consideration.

REFERENCES

1. Rubin RH, Young LS, Hansen WP, et al. Specific and nonspecific imaging of localized fisher immunotype 1 pseudomonas aeruginosa infections with radiolabeled monoclonal antibody. *J Nucl Med* 1988; 29:651–656.
2. Rubin RH, Fischman AJ, Nedelman M, Strauss HW. The use of radiolabeled, nonspecific, polyclonal hu-

man immunoglobulin in the detection of focal inflammation by scintigraphy: comparison with gallium-67 citrate and technetium-99m-labeled albumin. *J Nucl Med* 1989; 30:385–389.
3. Fischman AJ, Rubin RH, Khaw BA, et al. Detection of acute inflammations with ^{111}In-labeled nonspecific polyclonal IgG. *Semin Nucl Med* 1988; 18:335–344.
4. Trentham DE, Townes AS, Kang AH. Autoimmunity to type II collagen: an experimental model of arthritis. *J Exp Med* 1977; 146:857–868.
5. Rogers MP, Trentham DE, McCune WJ, et al. Effect of psychological stress on the induction of arthritis in rats. *Arth Rheum* 1980; 23:1337–1342.
6. Coulfield JP, Hein A, Dynesius-Trentham R, Trentham DE. Morphologic demonstration of two stages in the development of type II collagen-induced arthritis. *Lab Invest* 1982; 46:321–343.
7. Feitsma RIJ, Blok D, Wasser MNJM, Nieuwenhuizen W, Pauwels EKJ. A new method for ^{99m}Tc-labeling of proteins with an application to clot detection with an antifibrin monoclonal antibody. *Nucl Med Commun* 1987; 8:771–777.
8. De Sousa M, Bastos AL, Dynesius-Trentham A, et al. Potential of indium-^{111} to measure inflammatory arthritis. *J Rheumatol* 1986; 13:1108–1116.
9. Streule K, de Schrijver M, Fridrich R. ^{99}Tc^{m}-Labeled HSA-nanocolloid versus ^{111}In oxine-labeled granulocytes in detecting skeletal septic process. *Nucl Med Commun* 1988; 9:59–67.
10. Breedveld FC, Trentham DE. Progress in the understanding of inducible models of chronic arthritis. *Rheumatic Disease Clin North Am* 1987; 13:531–544.
11. Green FA. Joint scintiscans: present status. *J Rheumatol* 1979; 6:370–373.
12. Rosenspire KL, Blau M, Kennedy AC, Green FA. Assessment and interpretation of radiopharmaceutical joint imaging in an animal model of arthritis. *Arth Rheum* 1981; 24:711–716.