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Synthesis of ^{99m}Tc-HYNIC-interleukin-12, a new specific radiopharmaceutical for imaging T lymphocytes

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Abstract. *Purpose:* Few radiopharmaceuticals have been described for the study of lymphocyte trafficking despite its high clinical relevance. The main difficulty resides in the identification of a suitable highly specific probe to target these cells. Interleukin-12 (IL12) is a heterodimeric cytokine which plays a key role in the development of Th_1 lymphocytes. The aims of the present study were to label IL12 with ^{99m}Tc, to evaluate its ability to bind to activated T lymphocytes in vitro and to study its biodistribution in normal mice and mice affected by autoimmune colitis.
Methods: IL12 was derivatised with HYNIC-NHS and labelled with ^{99m}Tc. An in vitro binding assay was performed on KIT225 cells, an IL12 receptor-positive cell line. ^{99m}Tc-IL12 biodistribution in normal mice was studied. Targeting experiments were performed in Balb/c mice injected with KIT225 cells and in mice with chemically induced chronic colitis.
Results: ^{99m}Tc-IL12 labelling efficiency ranged between 75% and 85%. Saturation binding analysis revealed a K_d of 2.09 n*M*. Results of biodistribution studies showed a predominant hepatic route of excretion. A significant degree of uptake in the spleen and thymus was also observed. In mice injected with KIT225 cells, ^{99m}Tc-IL12-specific uptake in these cells increased over time. ^{99m}Tc-IL12 also accumulated significantly in bowel of mice affected by TNBS-induced colitis showing T lymphocyte infiltration at histology, while accumulation in colon from control animals was negligible.
Conclusion: We conclude that this radiolabelled cytokine is a suitable candidate for specific in vivo imaging of T lymphocytes: a step forward in molecular imaging of immune-mediated processes.

Keywords: Interleukin-12 – T lymphocytes – HYNIC – Molecular imaging

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Introduction

The diagnosis of occult chronic inflammation and infection is still a major clinical problem. Furthermore, so far no tools are available to correctly evaluate, non-invasively, the extent and the state of activity of chronic inflammatory diseases, nor are there any to evaluate the efficacy of immune therapies. It is therefore relevant for diagnostic, prognostic and therapeutic purposes to be able to develop new approaches that are able to identify, quantify and monitor the local immune response. Interleukin-12 (IL12) is a heterodimer of 75 kDa (p75) formed by two covalently linked glycosylated chains of approximately 40 kDa (p40) and 35 kDa (p35) [1, 2]. IL12 has a pivotal role in the induction of T helper-1 (Th_1) cell development, acting in antagonism with other cytokines (IL4, IL10) that favour differentiation of Th_2 cells [3]. The Th_1 subset is responsible for classical cell-mediated function and characterises a more "aggressive" pattern of inflammation leading to tissue destruction. The major secreting cells are monocytes, B cells and other MHC class II-positive cells. The presence of IL12 receptor can be detected only on activated T or NK cells [4], and analysis of IL12 binding

sites on phytohaemagglutinin-activated peripheral blood mononuclear cells has identified three binding sites with affinities of 5–20 pmol/l, 50–200 pmol/l and 2–6 nmol/l [5]. Although the IL12 receptor is also expressed on naive T CD4+ cells (Th_0), it becomes upregulated during Th_1 differentiation, in particular owing to the effect of interferon gamma (IFN-γ) stimulation, which also inhibits the growth of Th_2 cells [6].

Due to its ability to potentiate cell-mediated immunity, recombinant IL12 is currently being studied in clinical trials for the treatment of cancer [7] and chronic hepatitis [8]. The specific aims of this study were to label IL12 with ^{99m}Tc, to evaluate its ability to bind to activated T lymphocytes in vitro and to test its imaging potential in two relevant animal models.

Materials and methods

Preparation of HYNIC-conjugated IL12

Recombinant human IL12 was provided by Wyeth Research (Cambridge, MA) in the form of a frozen solution in water at a concentration of 4.5 mg/ml. Succinimidyl-6-hydrazinopyridine-3-carboxylate (HYNIC-NHS) was synthesised and conjugated to the IL12 as previously described by Abrams et al. [9], with slight modifications. Five microlitres of 1 mol/l $NaHCO_3$, pH 8.3, was added to 45 µl IL12 (4.5 mg/ml) in a 1.5-ml light-shielded vial. Subsequently, HYNIC-NHS (5 mg/ml) in dry dimethyl sulphoxide was added to the mixture. In order to assess the best conjugation condition, different molar ratios of IL12:HYNIC (1:1–1:10) and different reaction times (30–240 min) were tested.

HYNIC-IL12 was then purified from free HYNIC on a G50 microspin column (Amersham Biosciences Europe GmbH). Determination of IL12-HYNIC concentration was performed by comparing UV adsorbance at 280 nm with standard IL12 amounts. To determine the number of HYNIC molecules incorporated into the IL12 molecule, the hydrazino groups were converted to the corresponding hydrazone by reaction with benzaldehyde-2-sulphonic acid (Sigma-Aldrich). The absorbance of different HYNIC-IL12 preparations was read at 343 n*M* and compared with a standard curve [10]. HYNIC-IL12 was analysed by high-performance liquid chromatography (HPLC) using a size-exclusion Biosep SEC-3000 column (Phenomenex, Macclesfield, UK) eluted at 0.5 ml/min with 0.1 *M* phosphate buffer pH 7. HYNIC-IL12 aliquots were stored at −20°C.

Labelling and analysis of HYNIC-IL12

Three micrograms of HYNIC-IL12 were incubated with 10 µl tricine solution (100 mg/ml in 1 *M* acetate buffer pH 5.5), 0.1–0.2 ml $^{99m}TcO_4^-$ solution (1–10 mCi) and 10 µl of stannous tartrate (10 mg in 2 ml nitrogen-purged 0.1 *N* HCl) for 30 min at room temperature. All preparations were analysed using instant thin-layer chromatography–silica gel (ITLC-SG) chromatographic strips (Pall Corporation), either with acetone as the mobile phase for determination of the percentage of $^{99m}TcO_4$ or with saline for determination of the labelling efficiency. Insoluble and "colloidal" species were also identified on albumin-soaked ITLC strips using a solution of $EtOH:NH_4OH:H_2O$ (2:1:5). ^{99m}Tc-IL12 was then separated from non-bound ^{99m}Tc on a G50 microspin column (Amersham Biosciences). ^{99m}Tc-labelled IL12 was analysed by size-exclusion HPLC as described above. Electrophoresis of recombinant IL12, HYNIC-modified IL12 and ^{99m}Tc-IL12 using 10–15% gradient SDS-PAGE was performed on a PHAST system (Amersham-Pharmacia). Gels were first dried and then analysed by digital autoradiography on a Packard Cyclone phosphor imaging system (Perkin-Elmer, Ohio, USA).

In vitro stability of ^{99m}Tc-IL12

The stability of ^{99m}Tc-IL12 was evaluated both in saline and in human serum at 37°C at different time points (0–24 h) by ITLC-SG, using saline as the mobile phase. ^{99m}Tc-IL12 integrity and serum protein binding were tested by gel-filtration HPLC at 0.5, 1, 2 and 4 h after labelling.

Receptor binding assays

The ability of labelled IL12 to bind to its specific receptor was assessed in vitro by saturating binding assays on KIT225 cells, a cell line that constitutively expresses the IL12 receptor [11]. Briefly, for binding experiments, 30 µl of ^{99m}Tc-IL12 at increasing concentrations (from 10^{-12} to 10^{-8} *M*) were incubated in duplicate with 5×10^5 KIT225 cells (50 µl) alone or in the presence of a molar excess of the unlabelled cytokine (20 µl, 10^{-6} *M*) to saturate the specific receptor binding. Binding assay was performed at 4°C to avoid internalisation of the ligand, for 60 min. At the end of the incubation period, cells were harvested and centrifuged (at 9,000 *g* for 2 min) in a 500-µl vial, through a 200-µl layer of a mixture of mineral light oil:dibutyl phthalate oil (12.5:87.5). After centrifugation, vials were immediately frozen in N_2 and cut in half. Cells and supernatants were then counted separately for radioactivity [12].

Animals

For all animal studies, C57BL/6 SPF 10- to 12-week-old male mice from Charles River Laboratories were used. Mice were anaesthetised with ketamine sulphate (200 mg/kg) by intraperitoneal (i.p.) injection and additional light anaesthesia with ether. Temperature and relative humidity were continuously monitored; food (RF21diet by Mucedola) and water were given ad libitum. All animal studies were carried out in compliance with the local Animal Ethical Committee and the laws.

Biodistribution in normal mice

In normal Balb/c mice, 50 µCi of ^{99m}Tc-IL12 was injected via the retro-orbital vein. Groups of four mice were sacrificed at different time points after cytokine injection (10 min and 1.5, 3 and 6 h). Major organs and tissues were removed, weighed and counted for radioactivity (blood, lungs, thymus, liver, spleen, stomach, kidneys, small and large bowel) in a gamma-well counter. Results were expressed as % of injected activity per gram of tissue (%IA/g).

^{99m}Tc-IL12 targeting in mice bearing KIT225 cells

Balb/c mice were subcutaneously injected in the left flank with 6×10^6 KIT225 cells dissolved in 200 µl of an extracellular matrix of a mouse sarcoma solution (Matrigel) and in the right flank with 200 µl of Matrigel alone. After i.v. injection of 200 µCi of ^{99m}Tc-IL12, groups of three mice were sacrificed at different time points (1.5, 3 and 6 h), and the Matrigel pellets were removed, weighed and counted for radioactivity in a gamma-well counter. Results were expressed as %IA/g.

Induction of chronic colitis in mice

A model of chronic Th_1-mediated autoimmune colitis was induced in mice as previously described [13]. Briefly, using a 3.5F catheter, 100 μl of a solution of the hapten reagent trinitrobenzene sulphonic acid (TNBS) dissolved in 50% EtOH/50% PBS (2.5 mg) was instilled into the mouse rectum. As controls, mice injected with 50% EtOH/50% PBS were used. For the histological evaluation, colon specimens were fixed in 10% neutral-buffered formalin solution and embedded in paraffin, cut into 3-μm-thick sections, and stained with haematoxylin and eosin. Stained sections were examined for evidence of colitis using as criteria: the presence of lymphocyte infiltration, elongation and/or distortion of crypts, ulceration and thickening of the bowel wall. The degree of inflammation and lymphocytic infiltration was graded semi-quantitatively from 0 to 4 (0: no evidence of inflammation; 1: low level of lymphocyte infiltration with infiltration seen in <10% high-power fields (hpf), no structural changes observed; 2: moderate lymphocyte infiltration with infiltration seen in 10–50% hpf, bowel wall thickening, no evidence of ulceration; 3: marked degree of lymphocyte infiltration seen in >50% hpf, transmural infiltration with ulceration, no necrosis; 4: marked degree of lymphocyte infiltration seen in >50% hpf, transmural infiltration with ulceration, massive necrosis.

^{99m}Tc-IL12 targeting in mice with chronic colitis

Targeting studies were performed 5 days after the TNBS instillation, when a chronic lymphocyte-mediated inflammation had developed. Six mice were sacrificed 3 h after injection of 200 μCi of ^{99m}Tc-IL12, and the large bowel was removed, weighed, counted and imaged on a gamma-camera. Based on macroscopic and gamma-camera evaluation, affected segments were separated from the rest of the organ and weighed. The stool and the intestinal wall of the inflamed colon were separately analysed to calculate the percentage of excreted activity. The affected colon was then divided into 3-mm consecutive segments. Each segment was weighted, counted for radioactivity and histologically evaluated. ^{99m}Tc-IL12 uptake in each segment, expressed as %IA/g tissue, was correlated with the calculated inflammatory score on the same tissue sample.

Statistical analysis

Averaged uptake values are given as %IA/g±SD. Graph-Pad Prism Software was used for data analysis of in vitro binding assays. K_d (mean and 95% confidence interval range) was directly calculated by Graph-Pad Prism Software using a non-linear regression fitting. The Spearman rho correlation test was performed to correlate ^{99m}Tc-IL12 uptake in colon segments with histological scores. The Mann-Whitney *U* test was performed to evaluate differences in ^{99m}Tc-IL12 uptake in colon segments within histological scores.

Results

Preparation of HYNIC-conjugated IL12

Best results were obtained by incubating IL12 with HYNIC for 2 h using a 6:1 HYNIC/IL12 molar ratio. Under these conditions, a mean of 1.9 moles of HYNIC per mole of IL12 was incorporated. These conditions were used for all in vitro and in vivo experiments. After HYNIC conjugation, some dimerisation of IL12 was observed on HPLC and SDS-PAGE (Fig. 1).

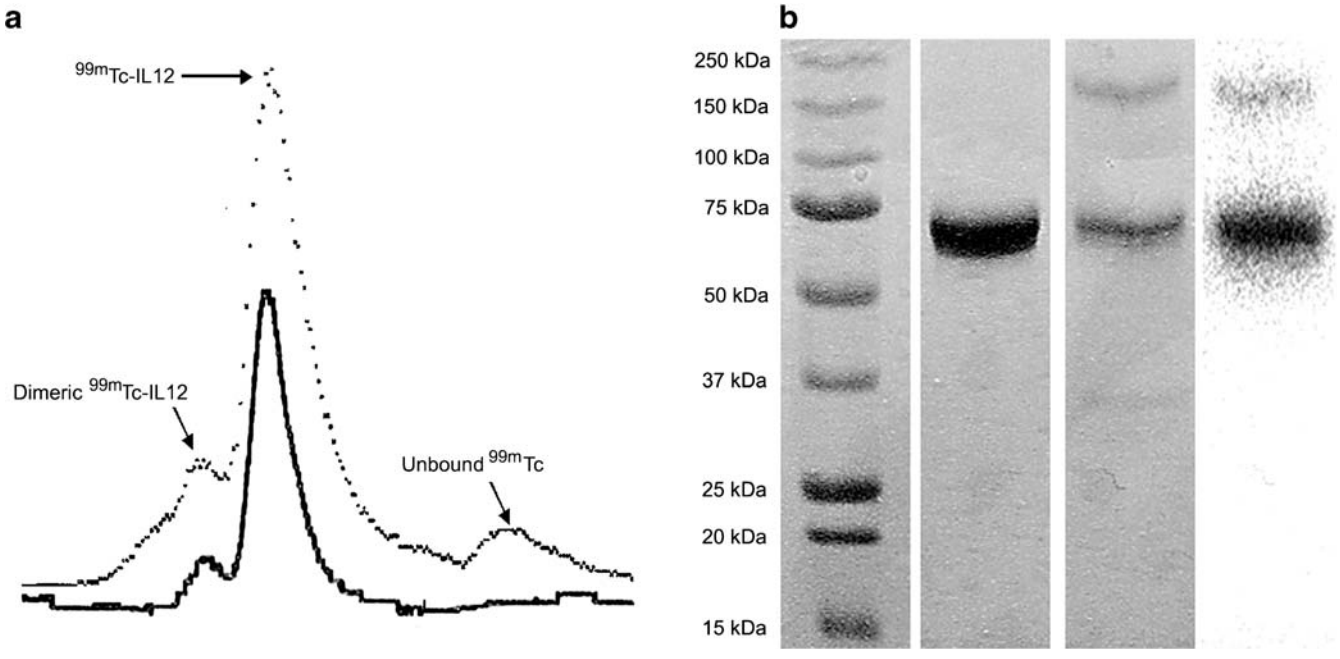

Fig. 1. Chemical characterisation of ^{99m}Tc-IL12 by gel-filtration HPLC (**a**) and SDS-PAGE (**b**). In **a**, both the UV chromatogram (*continuous line*) and the radiochromatogram (*dotted line*) are represented (retention times: 15 min for dimeric ^{99m}Tc-IL12 and 16.5 min for ^{99m}Tc-IL12). In **b**, the different tracks (*from left to right*) correspond to protein markers, recombinant IL12, conjugated IL12 and ^{99m}Tc-IL12

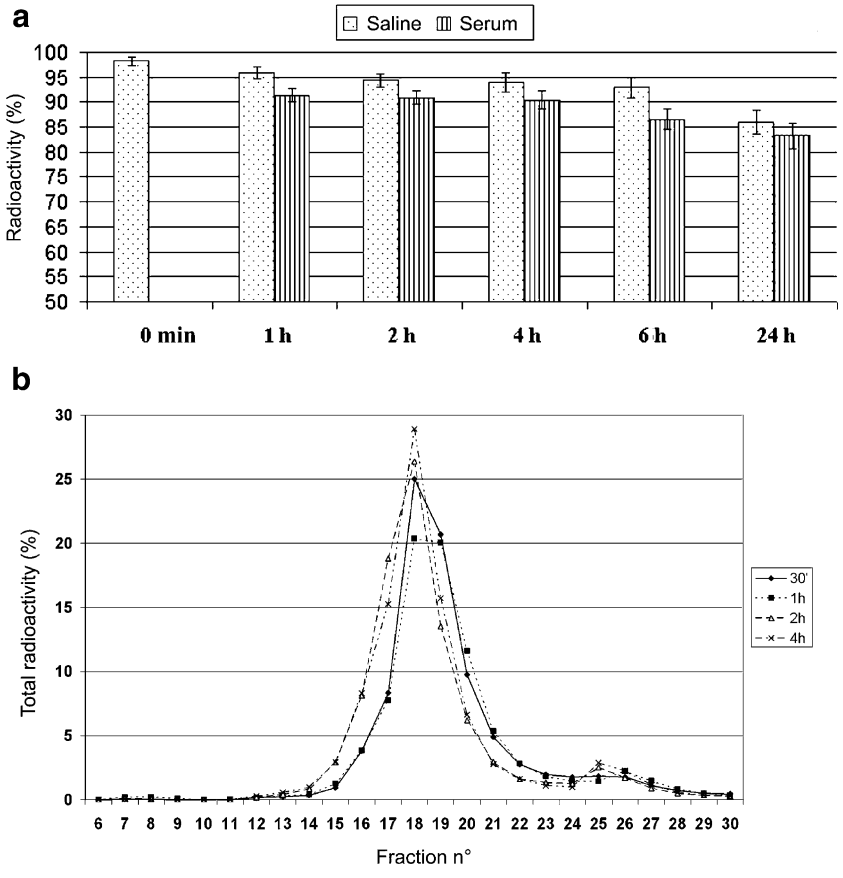

Fig. 2. Analysis of ^{99m}Tc-IL12 stability in saline and serum. **a** Percentage of IL12-associated radioactivity at different times in saline and human serum. **b** Evaluation of ^{99m}Tc-IL12 serum protein binding by size-exclusion HPLC. The radioactivity peak does not change with time, indicating that ^{99m}Tc-IL12 does not interact with serum proteins

Labelling and analysis of HYNIC-IL12

^{99m}Tc-IL12 labelling efficiency ranged between 75% and 85% with a maximum specific activity of 1.9 mCi/μg. The percentage of "colloids" was always below 5%. After G50 purification, the radiochemical purity was >97%.

^{99m}Tc-IL12 stability in serum and saline

Results of stability studies revealed a loss in purity of only about 10% in 6 h when the ^{99m}Tc-IL12 was incubated at 37°C in serum or in saline (Fig. 2a). Analysis of HPLC fractions showed no significant degree of ^{99m}Tc-IL12 binding to serum proteins (Fig. 2b).

Binding assays

Results of binding assays showed that after labelling IL12 retains the ability to bind to its specific receptor (Fig. 3). The calculated mean affinity constant (K_d) was 2.09×10^{-9} *M*, ranging from 6.23×10^{-10} to 3.50×10^{-9} (95% confidence interval).

Biodistribution in normal mice

Results of biodistribution studies showed predominant hepatic uptake. Significant uptake in the spleen and thymus was also observed (Fig. 4).

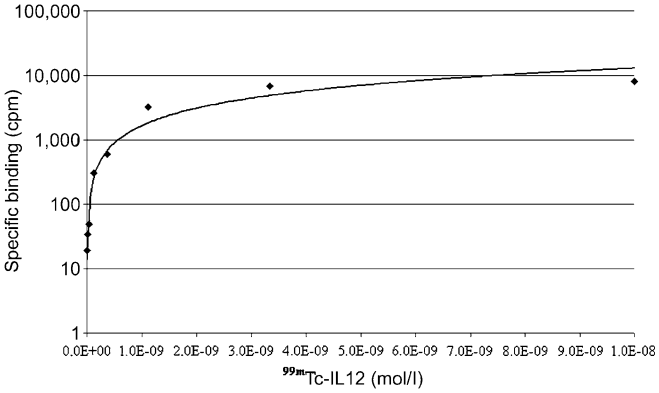

Fig. 3. Binding assay of ^{99m}Tc-IL12 on KIT225 cells. The data shown represent the specific binding to cells obtained after subtraction of non-specific binding from total binding

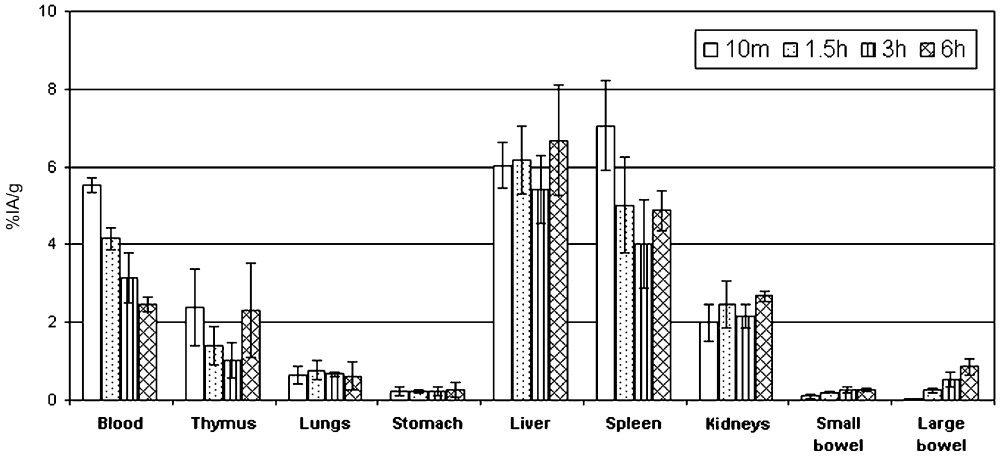

Fig. 4. Biodistribution of ^{99m}Tc-IL12 in normal mice (n=16) after 10 min and 1.5, 3 and 6 h from i.v. injection. Results are expressed as % of injected activity per gram of tissue (mean±standard deviation)

^{99m}Tc-IL12 targeting in mice injected with KIT225

^{99m}Tc-IL12 uptake by KIT225 increased with time and to a greater extent compared with Matrigel alone, with a maximum left to right ratio of 1.94 at 6 h (Fig. 5).

Induction of chronic colitis in mice

Whereas control mice, killed 5 days after administration of 50% ethanol solution, had no macroscopic lesions in the colon, severe colitis was observed in more than 80% of mice after administration of TNBS. In treated mice, the induction of colitis was indicated by a reduction of more than 30% in body weight. A total of 37 3-mm bowel rings were obtained from the cleavage of affected colon segments (as identified macroscopically). As expected from the patchy distribution of chronic inflammation described for this colitis, not all the rings analysed showed the presence of chronic inflammation. Histological evaluation of these specimens revealed a variable degree of colitis: score 0, n=6; score 1, n=6; score 2, n=7; score 3, n=6; score 4, n=12.

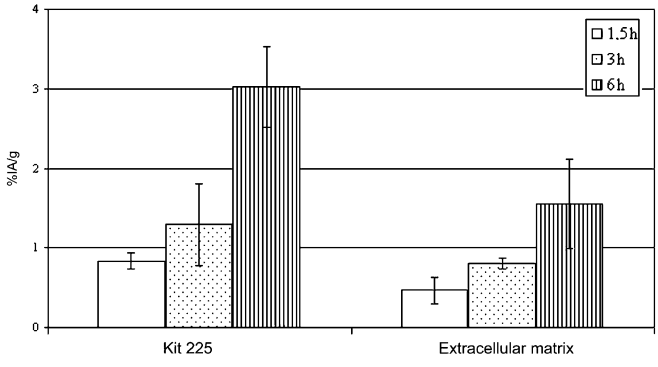

Fig. 5. In vivo ^{99m}Tc-IL12 uptake on KIT225 cells injected in the mouse flank, as compared to Matrigel binding as control, at different time points

^{99m}Tc-IL12 targeting in mice with chronic colitis

In mice with TNBS-induced chronic colitis, ^{99m}Tc-IL12 specifically accumulated in areas of chronic inflamed colon, while no significant uptake was detectable in non-inflamed areas or in the colon of control mice injected with 50% ethanol (Figs. 6, 7 and 8). The degree of ^{99m}Tc-IL12 uptake correlated with the degree of bowel lymphocytic infiltration at histology (r=0.801; p<0.01, Fig. 9). The individual counting of bowel and stool radioactivity

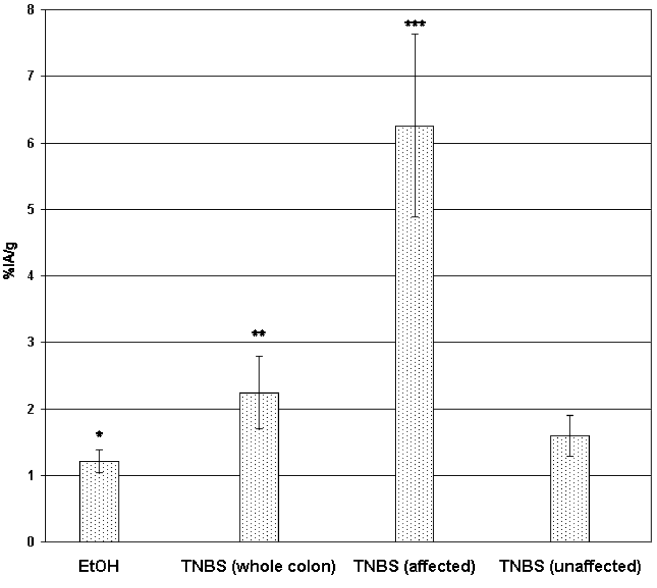

Fig. 6. In vivo ^{99m}Tc-IL12 uptake by the colon in control animals (injected with 50% EtOH) and in mice with TNBS-induced colitis. Due to the patchy distribution of chronic colitis, the specificity of ^{99m}Tc-IL12 uptake became more evident when affected and non-affected colon segments were individually evaluated. *EtOH 50% vs TNBS (whole colon) (p=0.014), TNBS (affected) (p=0.021), TNBS (unaffected) (p=0.043); ** TNBS (whole colon) vs TNBS (affected) (p=0.0014), TNBS (unaffected) (p=0.027); *** TNBS (affected) vs TNBS (unaffected) (p=0.021)

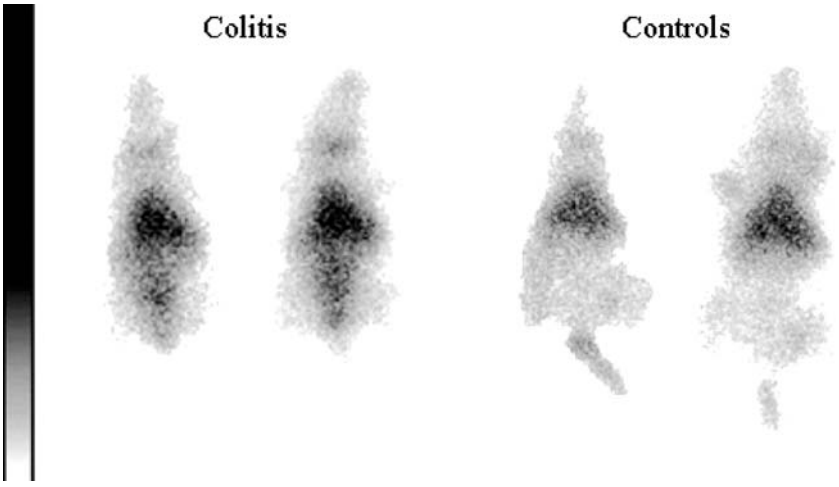

Fig. 7. Scintigraphic images of mice affected by TNBS-induced colitis (*left*) and of control mice (*right*) obtained 3 h after the i.v. injection of 200 μCi of ^{99m}Tc-IL12. A different degree of ^{99m}Tc-IL12 uptake can be observed in the abdomen of mice affected by colitis, while no significant accumulation is detectable in controls

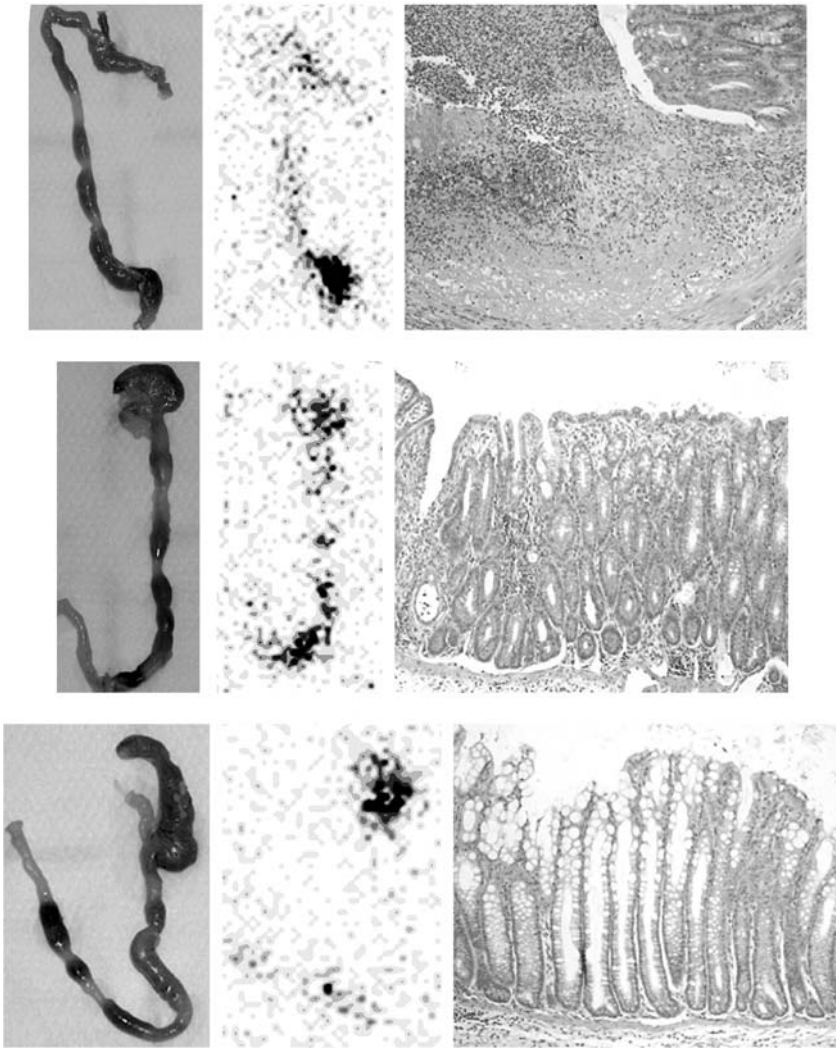

Fig. 8. Macroscopic views, scintigraphic images and histological characterisation (*from left to right*) of colons affected by TNBS-induced colitis (*upper and middle panels*) with histological scores of 4 and 2, respectively, and of a control colon (*lower panel*)

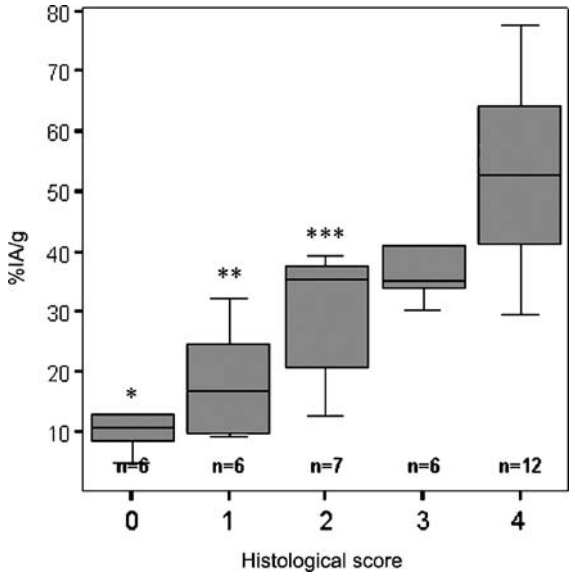

Fig. 9. Relationship between ^{99m}Tc-IL12 uptake on 3-mm colon segments and the histological score. *Group 0 vs group 2 (p=0.01), group 3 (p=0.004) and group 4 (p=0.001). **Group 1 vs group 3 (p=0.006), 4 (p=0.001). ***Group 2 vs group 4 (p=0.022)

showed that the contribution of stools to the total activity was 10.4±7.5% in affected and 9.2±3.2% in non-affected segments.

Discussion

The study of lymphocyte traffic in man has long been a goal for molecular nuclear medicine. ^{111}In-labelled autologous lymphocytes have been used in the past for the imaging of chronic diseases, but they have major limitations in that lymphocytes are very sensitive to radiation damage and the maximum specific activity tolerated is only 20 μCi×10^8 cells [14]. More recently it has been demonstrated that the labelling of lymphocytes using ^{99m}Tc-HMPAO does not significantly impair cell function, but the labelling efficiency is very low, with the result that the scintigraphic images are of poor quality [15]. A second limitation is due to the fact that in chronic inflammation an in situ clonal expansion of infiltrating lymphocytes occurs, rather than a migration from peripheral blood. Positron emission tomography with ^{18}F-fluorodeoxyglucose has been proposed for imaging chronic inflammatory disorders such as sarcoidosis [16], tuberculosis [17] and atherosclerosis [18] owing to enhanced glucose uptake by activated leucocytes, particularly macrophages. However, despite the high sensitivity of PET studies, ^{18}F-FDG lacks specificity owing to the nature of its means of accumulation.

A number of peptide radiopharmaceuticals have also been used for imaging chronic inflammation, including octreotide [19], substance P [20] and tuftsin antagonists [21], but with generally poor results and limited clinical use. Nevertheless, the potential clinical utility of a radiopharmaceutical for imaging chronic, lymphocyte-mediated inflammation is very high [22]. Such a radiopharmaceutical could be useful for evaluation of disease activity in chronic inflammatory disorders, allowing selection of the best therapeutic option and follow-up of its efficacy.

To this end, we have previously labelled interleukin-2 (IL2) with both ^{123}I and ^{99m}Tc [12, 23]. We now routinely use technetium-labelled IL2 for the in vivo diagnosis of chronic inflammation, and in particular to evaluate the severity and extent of organ-specific autoimmune diseases (as previously published with ^{123}I-IL2 [24, 25]) and to detect peritumoural lymphocytic infiltration [26]. Although radiolabelled IL2 is a valuable radiopharmaceutical for diagnostic purposes, the labelling procedure remains tedious, expensive and time consuming owing to the poor solubility and stability of this cytokine. We therefore wished to pursue the possibility of labelling a different cytokine able to bind to T lymphocytes. Interleukin-12 (IL12) is a cytokine that mediates several biological actions on T and NK cells, including IFN-γ induction, enhancement of cell-mediated cytotoxicity and co-mitogenic effects on resting T cells [1, 2]. The present work describes the synthesis and preclinical evaluation of ^{99m}Tc-IL12 and assesses its potential use for the specific visualisation of lymphocyte-mediated chronic disorders, particularly those characterised by a Th_1 pattern, which present an upregulation of the IL12 receptor. This study showed that IL12 can be efficiently labelled with ^{99m}Tc, using HYNIC as a bifunctional complexing agent. Biochemical analysis of ^{99m}Tc-IL12 performed by SDS-PAGE and HPLC showed that IL12 is not significantly altered by the conjugation and labelling process, except for some degree of dimerisation, which does not seem to interfere significantly with IL12 receptor binding. Nevertheless, analysis of binding assays showed a one-site like binding curve, although three IL12 receptors of differing affinity have been described in literature [5]. Dimerisation is consistent with literature data describing higher molecular weight species of labelled cytochrome c [27] and annexin V [28] at increasing numbers of introduced HYNIC moieties. The acceptable stability of IL12-associated radioactivity has been directly documented in vitro upon incubation in serum and saline, and indirectly in vivo, by the low stomach uptake observed in biodistribution studies.

In agreement with the in vitro findings, accumulation of ^{99m}Tc-IL12 in lymphocyte-rich tissues has been observed in both normal and pathological tissues. Biodistribution studies in normal mice indicated a predominantly hepatic accumulation with significant uptake in the spleen and thymus. The low accumulation of ^{99m}Tc-IL12 in the kidneys as compared with other radiolabelled cytokines is possibly related to its high molecular weight. The high accumulation in lymphoid organs suggests a possible specific IL12 binding on resident T lymphocytes. As seen with other high-molecular weight proteins, the activity cleared slowly from the blood but ^{99m}Tc-IL12 accumulation in inflamed areas became significant by 3 h after i.v. injection. ^{99m}Tc-IL12 specificity for T lymphocytes was demonstrated by its uptake by KIT225 cells, a T cell line

that constitutively expresses the IL12 receptor. In addition, the imaging potential of ^{99m}Tc-IL12 was also demonstrated in a more "physiological" model of inflammation, TNBS-induced chronic colitis, which closely resembles Crohn's colitis in humans. In this animal model, ^{99m}Tc-IL12 accumulated only in inflamed areas of the colon; no uptake was detectable in unaffected areas or in the colons of control animals. Histological evaluation of unaffected segments revealed in many tracts the presence of oedema and acute inflammation, possibly due to the chemical insult of TNBS and ethanol instillation. The absence of significant ^{99m}Tc-IL12 uptake in unaffected bowel segments underlines the lack of accumulation of the cytokine in inflamed tissues due to non-specific extravasation. As has been well demonstrated, the entity of non-specific uptake of labelled proteins (e.g. albumin nanocolloid) is much higher in acute inflammation than in chronic inflammation, owing to increased vascular permeability in the former. Results of biodistribution studies revealed that no significant bowel excretion occurred at least up to 6 h after the injection. We found that the activity in the small bowel is constantly low over time, while a slight increase in large bowel uptake was observed at late time points. The latter could be attributed to the ^{99m}Tc-IL12 uptake in the caecum, observed in vivo and ex vivo in all animals, possibly reflecting the physiological homing of lymphocytes in the appendix. The low uptake in the bowel and stools in both inflamed and normal colons suggests a possible use of ^{99m}Tc-IL12 for abdominal inflammatory disorders. Indeed, in patients with Crohn's disease, ^{99m}Tc-IL12 could be used to monitor the effect of treatment with monoclonal antibodies against IL12, which recently entered clinical trials [29]. However, a few considerations need to be borne in mind regarding the possible use of ^{99m}Tc-IL12 in humans. Although human recombinant IL12 is currently used as adjuvant therapy in oncological disorders, it is biologically active at doses as low as 300 ng/kg [30]. Thus, before considering the use of ^{99m}Tc-IL12 in humans, the preparation of ^{99m}Tc-IL12 should be further optimised. This could be achieved by increasing the specific activity of ^{99m}Tc-IL12, e.g. by purification of ^{99m}Tc-IL12 from non-labelled cytokine after the labelling reaction using a reversed-phase HPLC column. In this study we injected in mice a potentially toxic amount of IL12 without observing any side-effects, since human IL12 is able to bind to the murine IL12 receptor but is not biologically active in mice [31]. This can be explained by the homology of IL12 p40 chain between human and mouse IL12, which is primarily responsible for IL12 binding on its receptor [31]. By contrast, the difference between human and mouse IL12 p35, involved in the signal transduction, seems to be relevant in causing biological effects.

Conclusion

This study has demonstrated the potential of ^{99m}Tc-IL12 as a radiopharmaceutical for imaging of chronic inflammation. Owing to its high tropism for Th_1 lymphocytes, it could be particularly useful to specifically image this lymphocyte population, allowing "in vivo" monitoring of the effect of drugs that induce a shift from Th_1- to Th_2-mediated inflammation, by blockage of type 1 cytokines [32] or via clonal anergy induced by oral tolerance to antigens [33].

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