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Kinetic data of in-vivo labeled granulocytes in humans with a murine Tc-99m-labelled monoclonal antibody*

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Abstract. Twenty-five patients were examined in vivo with ^{99m}Tc labelled monoclonal antibodies; 15 with suspected infections with an antigranulocyte antibody (BW 250/183), 10 with suspected recurrence of a colorectal carcinoma with an anti CEA antibody (BW 431/26). Both antibodies were IgG1 isotypes. In the patients with suspected infections no change of the peripheral leukocyte count could be observed after the antibody injection (1 mg, n=9; 0.5 mg, n=1; 0.25 mg, n=6). In 2 patients examined with the anti CEA antibody (2 mg), a significant decrease of the peripheral leukocyte count could be observed. The recovery rate of the ^{99m}Tc antibody labelled granulocytes was calculated to be about 10%. The increase of the antibody-antigen binding was calculated to be 0.2%/min. In vivo the organ distribution curves demonstrated an increase of ^{99m}Tc activity over spleen and bone marrow of 1.1%/min, which was interpreted as antigen-antibody reactivity. The organ distribution curves of the anti granulocyte antibody over spleen and bone marrow showed typical binding characteristics to the local granulocyte epitopes. The curves over other organs showed a simple perfusion pattern. The curves of the anti CEA antibody showed a perfusion pattern over all the examined organs. A sham dialysis model in one patient with renal insufficiency undergoing regular dialysis treatment demonstrated the viability of ^{99m}Tc antibody labelled granulocytes in vivo. The kinetic patterns of the ^{99m}Tc antibody in patients with Crohn's disease were interpreted as CEA binding of the antibody in the bowel wall.

Key words: Tc-99m-antigranulocyte antibodies – Immunoscintigraphy – Kinetic data

The importance of ^{111}In-oxine or ^{99m}Tc-HMPAO labelled granulocytes in the diagnosis of inflammatory diseases and abscesses is based on the high sensitivity and specificity of this method (Becker et al. 1986; Coleman 1982; Loken et al. 1985), which is also reported for other methods such as ^{111}In-chloride (Sayle et al. 1983; Iles et al. 1987) or ^{67}Ga (Hauser and Alderson 1978), whose labelling mechanism is not yet completely understood. The main reason for the favorable role of in vitro labelled granulocytes is the fact that cells which are responsible for the inflammatory process are selectively labelled and are still viable after the labelling procedure as shown by activation in vivo comparable to non labelled cells (Becker 1988). So kinetic data are important to understand the new cell labelling methods, respectively of ^{123}I (Locher et al. 1986) or ^{99m}Tc antibody labelled granulocytes (Joseph et al. 1987).

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Patients and methods

Patients

Fifteen patients were examined with an ^{99m}Tc labelled anti granulocyte antibody MAB BW 250/183 (Behring-Werke, Marburg) after they had given their informed written consent. Ten patients suffered from a suspected infection of their hip prothesis, two patients had lung abscesses, one patient suffered from an infection of his polycystic kidneys and underwent regular dialysis treatment and two patients suffered from active Crohn's disease.

As controls for antibody cross reactivity, ten patients after surgical resection of a colorectal carcinoma were examined with the monoclonal antibody BW 431/26 (Behring-Werke, Marburg) because they were suspected to have local recurrence or metastases of their carcinoma.

Methods

Characterisation of the antibody. The ^{99m}Tc labelled monoclonal antigranulocyte antibody (BW 250/183) is an IgG1 isotype and detects a 180 kdalton glykoprotein (Bosslet et al. 1985). The recognized epitope is localized on CEA and NCA-95. It is reactive with approximately 90% of the peripheral granulocytes as is shown by cytofluorometric analysis and a variety of tissues such as colon, stomach, lung and breast (Bosslet et al. 1985) in immunohistochemical experimental models.

The ^{99m}Tc labelled anti CEA antibody used as a control antibody for kinetic studies is also an IgG1 isotype and detects a 180 kdalton epitope (Bosslet et al. 1985) which is located on the CEA glykoprotein. Therefore, MAB BW 431/26 does not bind to normal human granulocytes which express NCA-95 but not CEA.

Application of the antibody. The patients have all given their written informed consent to the study. The application of

the antibody was approved as a clinical trial by the Deutsches Bundesgesundheitsamt.

We gave no premedication to the patients as thyroid blocking agents or antihistaminic drugs, 2 mg of the anti CEA or 1 mg ($n=9$), 0.5 mg ($n=1$) or 0.25 mg ($n=5$) of the antigranulocyte antibody were prepared in 5 ml 0.9% saline and slowly injected during 5 min under permanent medical control by a physician.

Data acquisition. In all the patients who received the anti CEA antibody and in ten patients who received the antigranulocyte antibody, the gamma camera (GE 400 T), fitted with a general purpose collimator, was superimposed over the lungs, the heart, the liver and the spleen. In five other patients with known infections of the abdomen or the hip prosthesis the camera was superimposed over the abdomen or the pelvis. Over the first 30 min, a dynamic study (64 × 64 pixel matrix; 1 frame/min) was performed and the data were stored on-line to a computer system (DEC Gamma 11). The ascent of the displayed time activity curves of the different ROIs was calculated and expressed in % increase/min. Thirty-five min post injection, a static image was performed (64 × 64 pixel matrix/500000 cpm) of the region of interest, which was known by the result of a previously performed ^{111}In-oxine granulocyte scan. One h post injection, a whole body scan was acquired. Static gamma camera images of the whole body were performed at 4–5 h and 20 h post injection (64 × 64 pixel matrix; 500000 counts).

Blood collection and counting. Blood samples were taken from a cubital vein in all the antigranulocyte and anti CEA patients 5 min, 15 min, 30 min, 45 min and 60 min and in some patients also 4 h and 20 h after injection of the antibodies. In all the samples the peripheral leukocytes were counted by a standard procedure. The gamma activity was counted with a gamma counter.

Percoll®/plasma gradients. According to our previously published procedure (Becker et al. 1986), in every patient 40 ml peripheral venous blood was drawn twice for a discontinuous Percoll®/plasma gradient at different times after antibody injection. In this way 2 samples at 5, 15, 30, 45 and 60 min were taken from different patients. The activity distribution in the Percoll®/plasma gradient was measured by the gamma camera (128 × 128 pixel matrix). The activity distribution was roughly estimated by ROI technique over the different gradient layers.

Calculation of recovery. The recovery rate of granulocyte bound ^{99m}Tc activity was calculated according to the formula *recovery* = *activity*/ml × *blood volume* × 100/*injected activity*.

Sham dialysis model. The ^{99m}Tc antigranulocyte antibodies were injected into the supine patient who was prepared for sham dialysis. For this procedure 2 needles were placed into their arteriovenous fistula and a bolus injection of heparin (2000 IU) was given. The extracorporal circuit consisted of one blood pump and a capillary dialyzer. The dialyzer was equipped with a cellophane membrane (Cuprophane®). The blood passed through the dialyzer without dialysat flow or ultrafiltration.

The patients were supine under a gamma camera which was fitted with a parallel-hole, high-resolution, low-energy collimator (GE Maxi 400). On-line to a computer system (DEC Gamma 11) the granulocyte kinetic was registered over both lungs, the heart, the spleen and the liver for 60 min post injection (64 × 64 pixel matrix; 1 frame/min). The activity distribution patterns over these organs were registered and examined by the ROI technique. Twenty min after the antibody injection the dialysis was started and stopped 60 min after injection.

Five ml venous blood for leukocyte counting and measurement of ^{99m}Tc activity was taken 5 min prior to the start of the sham dialysis, immediately after antibody injection and at 5 min intervals. The peripheral leukocyte concentration was counted by a routine laboratory procedure, the ^{99m}Tc activity in the same samples was measured in a gamma counter.

Results

Antibody specificity

The ^{99m}Tc labelled antibody BW 250/183 was selectively reactive with granulocytes which were isolated by a Percoll®/plasma gradient. No reaction by other blood components could be detected in the gradient bands. The MAB BW 431/26 showed no reaction with granulocytes. All the activity was localised in the supernatant of the gradient.

Recovery of granulocyte bound activity

The blood activity disappearence of the patients and their mean value and standard deviation of the mean was calculated and expressed as the recovery (in %) of the injected activity.

The results of the Percoll®/plasma gradients showed a different distribution of the plasma free activity and cell bound activity dependant on the time after antibody injection (plasma free activity: 10 min 85%, 20 min 55%, 30 min 45%, 60 min 20%. Cellular bound activity: 10 min 15%, 20 min 45%, 30 min 55%, 60 min 80%). These data are the mean values of two measurements. The difference between the blood activity disappearence and the plasma free activity curve was called the cellular bound activity curve. The cellular bound activity increases from 3% (5 min p.i.) to about 10% (45 min p.i.) (Fig. 1). After 45 min a plateau phase could be measured and in some patients a decrease of cellular bound activity.

Peripheral leukocyte count after mab injection

The peripheral leukocyte count after injection of MAB BW 250/183 in different concentrations (1 mg, 0.5 mg, 0.25 mg) was stable and showed no significant decrease 5, 15, 30, 45 or 60 min p.i. Four h and 20 h after injection 5 patients the same peripheral leukocyte concentrations could be found (Fig. 2). The same could be observed in patients after MAB 431/26 injection (2 mg), but in 2 patients a decrease of the peripheral leukocyte count could be observed (Fig. 2).

Antibody organ distribution pattern after injection

The kinetic curves of MAB 250/183 over the lungs, the heart and liver showed a normal organ perfusion pattern with a rapid peak time and a rapid decrease after the peak

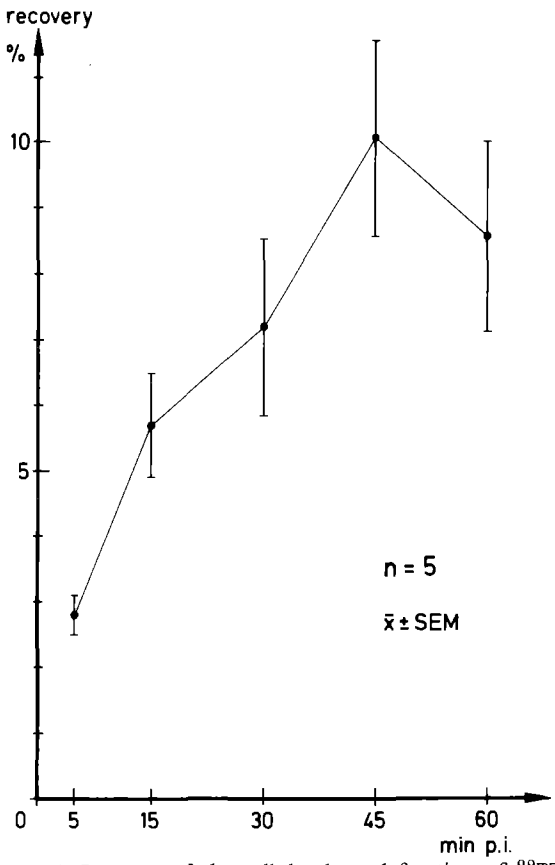

Fig. 1. Increase of the cellular bound fraction of ^{99m}Tc labelled antibodies with increasing time after injection. The data were calculated as the difference between blood activity disappearence curve and the clearence of plasma free activity

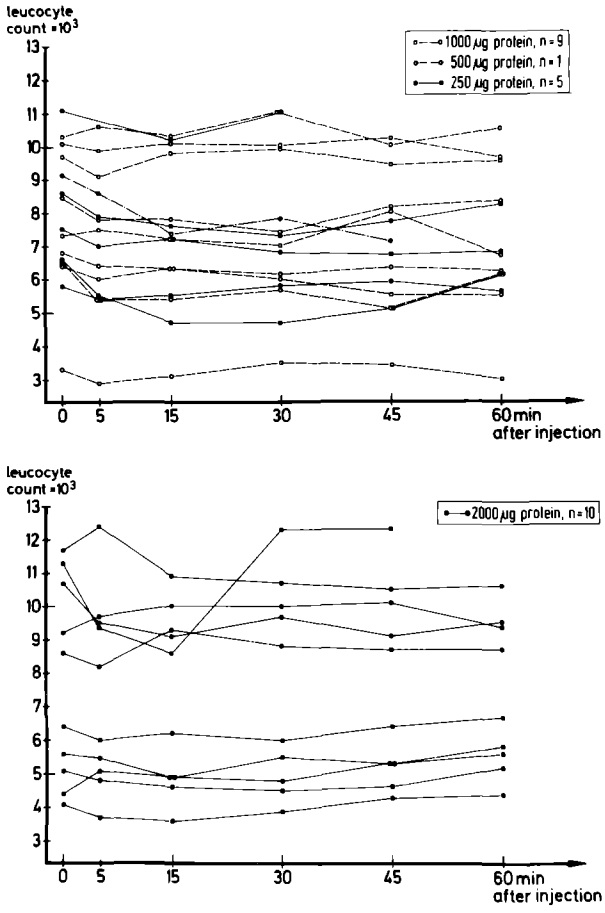

Fig. 2. Peripheral leukocyte count at different times after injection of different concentrations of the ^{99m}Tc labelled antigranulocyte antibody BW 250/183 (*top*) and of the ^{99m}Tc labelled anti CEA antibody BW 431/26 (*bottom*)

maximum. Afterwards the activity showed a plateau with only a faint decrease of the ^{99m}Tc activity (Fig. 3).

The curve over the spleen increased progressively from the end of the 5 min antibody injection period. The same curves and binding characteristics could be found over the bone marrow of the lumbar spine or the pelvis. The slope of the curves showed an increase of 1.1%/min of the initial activity. Using MAB BW 431/26, the comparable kinetic curves over the heart, lungs, liver, spleen and bone marrow were perfusion curves without typical binding characteristics in any of the examined organs (Fig. 3).

The sequential studies (1 frame/min) during the first 30 min after injection over different infectious sites (lung, bone) showed no binding of the antibody at the focus of infection, which was proven by a preceeding ^{111}In-oxine granulocyte scan. Only the patients with acute Crohn's disease showed hyperemia during the first 20 min after injection of the antibody. Afterwards no more increased activity could be localised over the infection, but all sites of infection could be localised 4 h p.i. and showed no change 20 h p.i.

Crohn's disease model

One female with an active Crohn's ileocolitis showed a pathologic activity in projection to the terminal ileum and the ascending colon. The late scan (20 h) was identical concerning the localisation but showed a two fold increase of activity in comparison to the 4 h scan (Fig. 4). The activity was compared by the region of interest technique with the bone marrow activity in the proximal femur. The same pattern showed the second female with active ileitis and with an abscess perforating to abdominal wall. The abscess showed the same pattern as the inflamed bowel segments.

Whole body scans

The whole body scans (1 h p.i.) of the patients who were examined with MAB BW 250/180 were comparable with a bone marrow scan. The MAB BW 431/31 scans showed a blood pool scan without bone marrow visualisation (Fig. 5).

Sham dialysis model (Fig. 6)

The peripheral leukocyte count in the patient decreased after the start of the sham dialysis (0 min $6.8 \times 10^3/\mu l$) significantly (15 min: $2 \times 10^3/\mu l$; 30 min: $2.99 \times 10^3/\mu l$) and nearly reached the initial level at 60 min ($5.98 \times 10^3/\mu l$).

The ^{99m}Tc activity in the peripheral blood samples decreased from 24×10^4 cpm/ml to 20×10^4 cpm/ml at 15 min p.i. and 13×10^4 cpm/ml at 30 min p.i. and increased once more at the end of the dialysis.

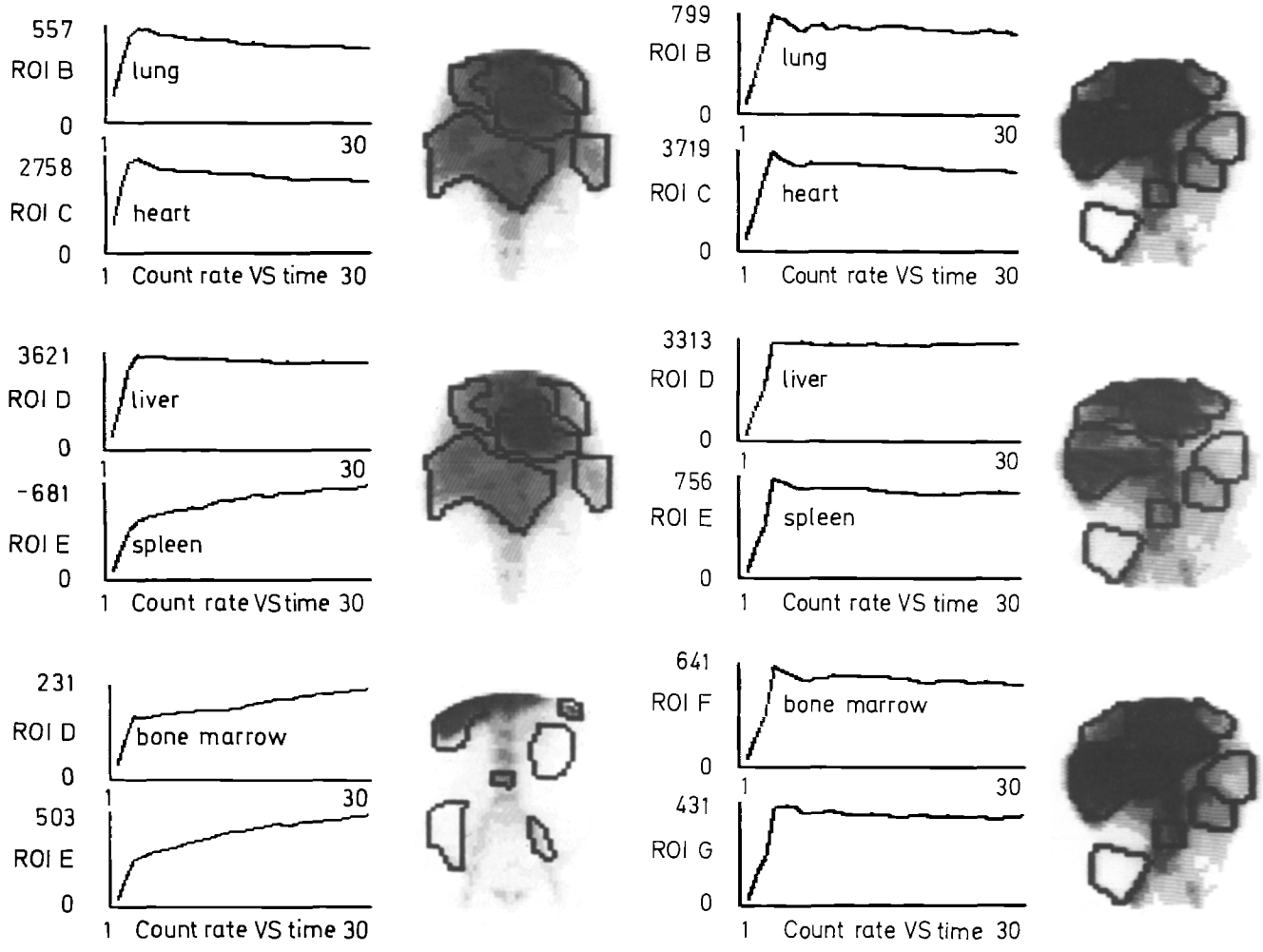

Fig. 3. ^{99m}Tc labelled antibody distribution pattern over different organs over the first 30 min after injection of the antibody (*left:* anti granulocyte antibody; *right:* anti CEA antibody)

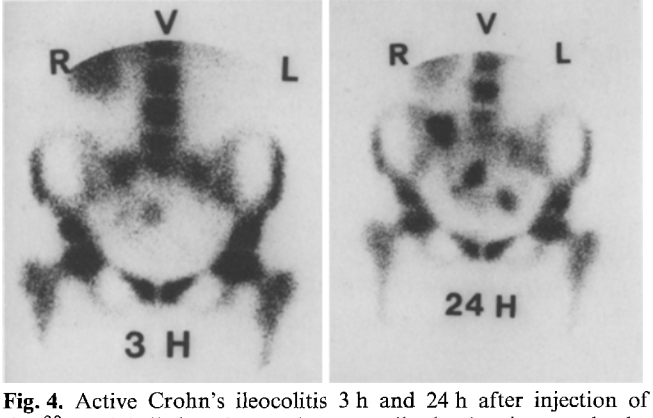

Fig. 4. Active Crohn's ileocolitis 3 h and 24 h after injection of the ^{99m}Tc labelled anti granulocyte antibody showing nearly the same activity pattern without any sign of faeces excretion and granulocyte transportion with the faeces. The images show an enhancement of the focus activity from 3 h to 20 h p.i.

In vivo, the count rate over the lungs and the granulocyte content of the lungs increased after the start of the hemodialysis and reached a maximum 15 min afterwards. The increase of lung activity was symmetric over both lungs. The spleen activity curve showed no continuous increase. At the moment of the increase of the lung activity, the spleen curve reached a plateau and increased once more beginning at the time of the decrease of the lung activity.

Discussion

The comparison of two ^{99m}Tc labelled antibodies against CEA was necessary to exclude unspecific migration patterns of antibodies. The specificity of MAB BW 250/183 and BW 431/26 was sufficiently tested in animal models (Bosslet et al. 1985). Our human in vivo kinetic studies and density gradient isolation of granulocytes prove the antigranulocyte specificity of MAB BW 250/183 and clearly demonstrate that there is no reaction with granulocytes of the antibody BW 431/26. The in vivo distribution patterns demonstrate a rapid binding of BW 250/183 with granulocytes in the bone marrow and spleen. Consequently the whole body scans are best bone marrow scans (Fig. 5). The kinetic patterns are comparable with the kinetics of ^{111}In-oxine or ^{99m}Tc-HMPAO labelled granulocytes (Becker 1988; Becker et al. 1988b). Only the first 5 min of each curve are significantly different. This is due to the slow injection of the antibody solution because of probable anaphylactic reactions of the patients.

In comparison, the MAB BW 431/26 demonstrates high blood pool activity with no specific organ binding or distribution pattern, as could be demonstrated in the dynamic

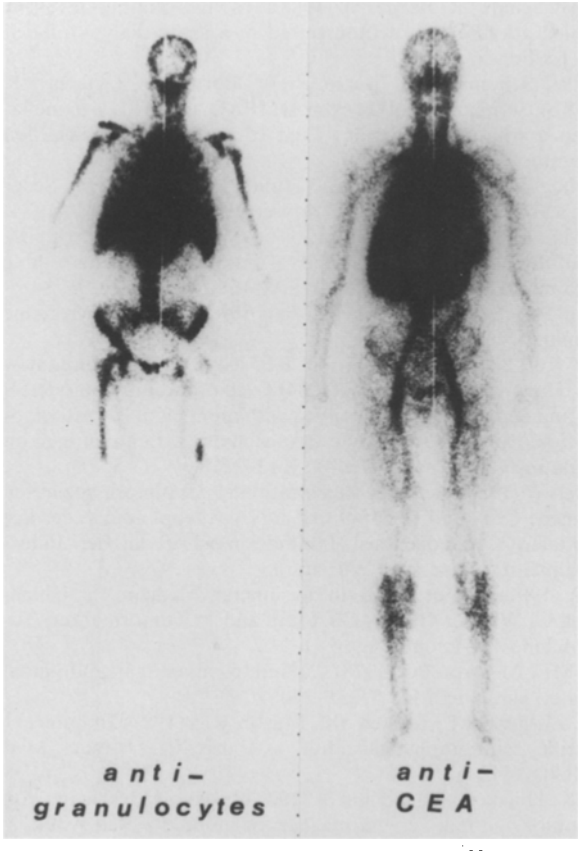

Fig. 5. Whole body distribution pattern of the ^{99m}Tc labelled anti granulocyte antibody BW 250/183 demonstrating a bright bone marrow scan and the ^{99m}Tc labelled anti CEA antibody BW 431/26 demonstrating a blood pool scan 1 h p.i.

studies after antibody injection (Fig. 3). So this antibody is not cross reactive to granulocytes in vivo.

Besides the sessile epitopes of granulocytes in the spleen and the bone marrow, the antibody also reacts with the circulating granulocytes. The recovery rate is not higher than 15% after 90 min. In comparison, the recovery rates of ^{111}In-oxine and ^{99m}Tc-HMPAO labelled cells is significantly higher (Becker 1988; Becker et al. 1988b). This might be the reason for the low focus to background ratio in the MAB scans in comparison to the high recovery rates in the standard methods (Fig. 1), because the availability of ^{99m}Tc antibody labelled granulocytes is significantly lower than the availability of ^{111}In-oxine or ^{99m}Tc-HMPAO labelled cells.

Dillman et al. (1984) reported that it seems imperative to rigorously exclude binding to circulating cells of all types for MABs with potential human application. All the circulating blood cells opsonised with MAB are rapidly removed from the circulation by the reticulo-endothelial-system, particularly liver and spleen. The consequence was a rapid decrease of the circulating granulocyte counts with system toxicity when Dillman et al. (1984) applied 5 mg protein. Our in vivo applicated antibody BW 250/183 was granulocyte specific and was an IgG1 isotype, but we did not find any significant decrease of the circulating granulocytes over the first 60 min, and in some patients over 24 h. In only 2 patients, who were examined with the BW 431/26, a significant decrease of granulocytes could be recognized. Perhaps this was due to the high protein dose of 2 mg antibody whereas in the granulocyte scans only 1 mg or less protein was used.

One of the central questions of the antigranulocyte antibodies is the question about the binding mechanism. It is interesting whether it is the antibody itself which labels the abscess or whether the antibody labelled cells migrate to the focus of infection. This essential question cannot be conclusively answered by our preliminary data, but we can explain some principle problems. The sham dialysis model clearly demonstrates that antibody labelled granulocytes behave in the same manor as ^{111}In-oxine labelled or unlabelled cells (Becker et al. 1988a, b). These data are only preliminary because in this study only one patient could be examined with this fascinating in vivo viability model. In comparison to the data with ^{111}In-oxine labelled granulocytes (Becker 1988), antibody labelled granulocytes can be activated by the cellophane membrane. This results in pulmonary sequestration after which the cells leave the lungs again. The mobilisation of granulocytes results in a depletion of ^{99m}Tc activity from the circulation (Fig. 6) and the spleen. The spleen curve in the ^{99m}Tc-MAB model is different from the ^{111}In-oxine model because the maximum of the spleen activity in the MAB scan is later than in the ^{111}In-oxine scans. So the MAB spleen curve only shows an interruption of the continuous ascending curve.

The ascent of the antibody curves over the bone marrow was calculated to be 1.1%/min. The estimated antibody granulocyte reaction in vivo was calculated 0.2%/min. These estimated binding kinetic data are in comparable

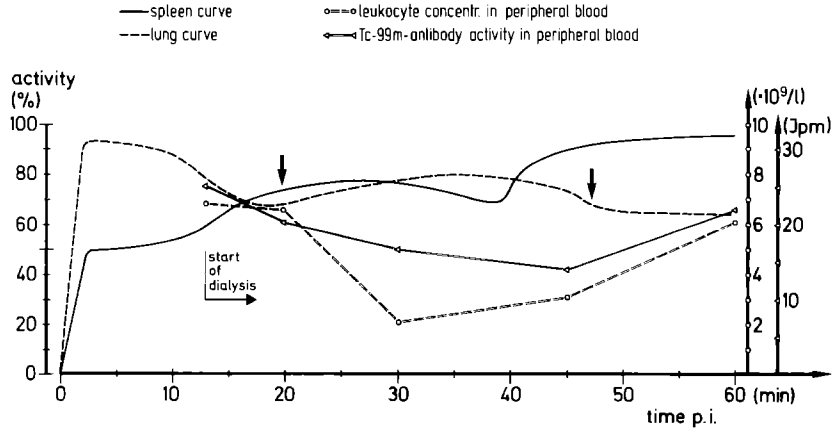

Fig. 6. Kinetics of leukocytes during dialysis. Schematical demonstration of peripheral ^{99m}Tc labelled and unlabelled granulocyte decrease, spleen depletion of ^{99m}Tc activity and lung sequestration of ^{99m}Tc activity after the start of the sham dialysis demonstrating the vivo viability of ^{99m}Tc antibody labelled granulocytes

ranges. The data explain the slow concentration of antibody labelled granulocytes in the focus. Negative scans in the first 30 min after antibody injection can be well explained by these results. Unlike ^{111}In-oxine or ^{99m}Tc-HMPAO, granulocyte scans demonstrate a 90% localisation of abdominal infections 30 min after cell reinjection (Becker et al. 1986).

One of the best kinetic models of in vivo granulocyte viability and vitality is the inflammatory bowel disease (Becker et al. 1986; Saverymuttu 1981). Thirty min p.i. most of the abdominal infectious lesions can be localised by a cell pooling in the focus. The granulocytes then migrate into the bowel wall (1–6 h) and into the bowel lumen and are transported by the faeces (20 h) (Becker et al. 1986). This typical pattern has not yet been observed in Crohn's disease or ulcerative colitis patients, who were examined with ^{99m}Tc or ^{123}I labelled antigranulocyte antibodies. The explanation of these data are at the moment still hypothetic but convincing. It is well known that the serum CEA concentration in about 30% of patients with Crohn's or ulcerative colitis is elevated (Gross 1981). On the other hand, the mucosal CEA content in Crohn's or ulcerative colitis is higher than in normal mucosa (Fischbach 1988). It is also proven in vitro that the MAB BW 250/183 reacts with normal colon mucosa in the histological sections (Bosslet et al. 1985). So the mechanism of Crohn's disease localisation might be the localisation of expressed CEA. However, it has to be noted that antibody labelled cells might be able to migrate in the circulation but not through the bowel wall. These possible mechanisms still have to be proven by further studies.

In conclusion, our data demonstrate that the ^{99m}Tc antibody labelled granulocytes in vivo have a comparable kinetic behaviour under normal and activated conditions. The delayed localisation of infections is due to a slow antibody antigen reaction. The Crohn's disease model demonstrates that besides a specific granulocyte labelling, a CEA labelling in the focus of infection also has to be discussed.

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