Human Antimurine Antibodies (HAMA) In Vivo Complex Formation and the Outcome of Immunoscintigraphy

TO THE EDITOR: Sakahara and colleagues (*1*) confirm the role of antibody complex formation on the clearance rate and biodistribution of intravenously administered murine monoclonal antibodies in patients undergoing serial studies. We would like, however, to comment on the occurrence of pre-existing HAMA and the effect of immune complexes on the outcome of immunoscintigraphy.

Although their study is small, the finding of pre-existing HAMA in one of the ten patients studied and the absence of the >900 kD complexes in the patient suggest that the pre-existing HAMA in the patient is IgG and consistent with a secondary immune response. We have estimated pre-injection HAMA levels in 20 patients undergoing immunoscintigraphy by a direct enzyme-linked immunosorbent assay (ELISA) using the ImmuSTRIP HAMA ELISA test system (Immunomedics, New Jersey). The mean preinjection HAMA level was 102 ± 0.40 ng/ml (range: 20–150 ng/ml) Eighteen (90%) of the patients had HAMA values >40 ng/ml, which we have taken as the upper limit of normal (the assay sensitivity is ~40 ng/ml). None of the patients we studied had known prior exposure to mouse proteins, and the absence of a positive skin reaction in 10 of the patients on whom skin tests were performed further demonstrates the uselessness of the skin test in detecting the presence of HAMA.

Several authors have described the occurence of endogenous HAMA (*2–5*), but the variety of the techniques used for measuring HAMA levels makes comparison of results and the estimation of normal values difficult. Although endogenous HAMA has been proposed to be a reflection of the normal immune system (*6*), its occurence or the presence of circulating or sequestered tumour-associated antigen could result in the formation of immune complexes on the first i.v. administration of murine monoclonal antibody for the purpose of immunoscintigraphy. Two of the patients we have studied

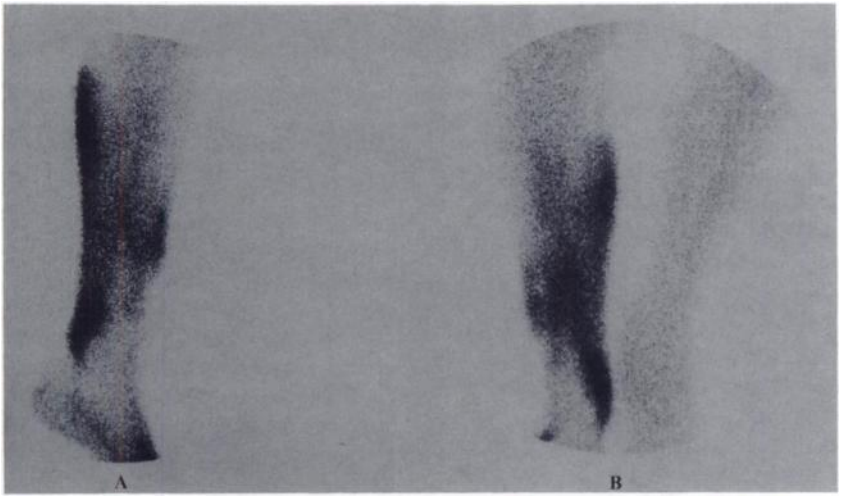

FIGURE 1
Scintigrams from a patient with suspected metastatic melanoma on whom combined immunoscintigraphy and immunolymphoscintigraphy were performed. Increased uptake in the right leg is probably due to antibody-antigen complexes in lymphatics. (A) Lateral view of right leg and (B) anterior view of both legs.

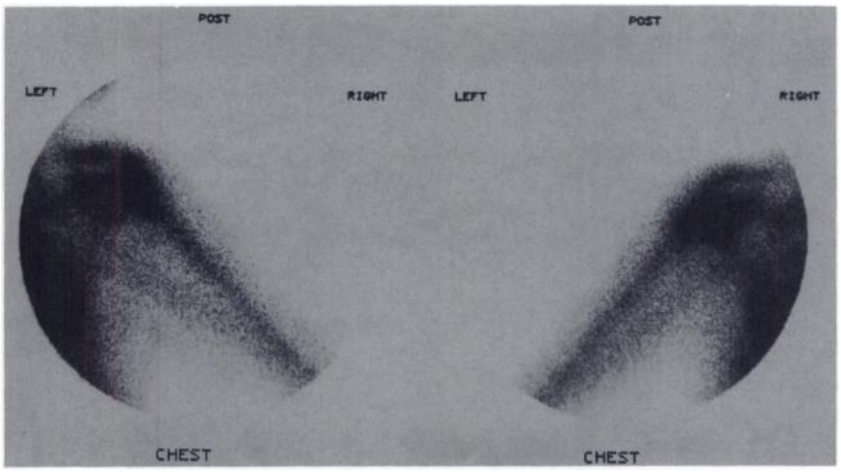

FIGURE 2
Scintigrams from a 79-yr-old patient 6-hr postintravenous injection showing bilateral increased uptake in the humerii and scapulae probably due to clearance of immune complexes in the reticuloendothelial system.

with ^{99m}Tc-labeled antimelanoma antibody F(ab')2 fragments, 225.28S, illustrate this point.

Patient 1 (Fig. 1) had combined immunolymphoscintigraphy and immunoscintigraphy 2 yr after the excision of a primary melanoma from her right ankle. There were no palpable lesions in the areas of increased uptake and the patient has yet to develop disease in these areas 14 mo after the study. The uptake is thought to be due to entrapped antibody-antigen complexes resulting from the binding of the antibody to sequestered antigen within the lymphatics. Patient 2 (Fig. 2) showed a pattern of uptake normally observed in patients receiving second or subsequent injection of murine monoclonal antibody and had increased uptake in the liver, spleen, and bone marrow. Since this patient had no known previous exposure to mouse IgG, it was thought that the pattern of uptake could be due to clearance of circulating immune complexes, caused by pre-existing HAMA, by the reticuloendothelial system.

While the formation of immune complexes, be they antibody-circulating-antigen or antibody-antibody, can alter the clearance rate and biodistribution of injected monoclonal antibodies they can contribute to the nonspecific uptake observed during early imaging times especially when radionuclides such as ^{99m}Tc are used. The immune complexes could be entrapped within the vasculature and disrupted architecture of tumours where the large intracapillary pores of newly grown capillary beds permit the extravasation of the complexes into the interstitial space. Increased macrophage activity, especially when there is associated tumour necrosis, may result in ingestion of the complexes. The immediate effect of these is an observed increased (nonspecific) uptake on early imaging (probably up to 24 hr). However, ingestion of the complexes by macrophages within the tumour results in rapid removal of the radiolabel, which results in the lower count rates, lower target-to-background ratios, and high false-negative results observed by many authors on late imaging. This is in addition to interference with tumour targeting by anti-idiotypic HAMA and the effects of faster clearance of the antibody.

The occurrence of pre-existing HAMA in some patients makes it desirable that this should be evaluated before murine monoclonal antibodies are administered for diagnostic purposes. The effect of circulating immune complexes should also be borne in mind when the choice of antibody radiolabel makes imaging beyond 24 hr impracticable.

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Right Ventricular Ejection Fraction by First-Pass Curve Oscillations

TO THE EDITOR: The high frequency sampled first-pass radioventriculogram consists of the low-frequency curve undulated by the successive spikes, each reflecting intra-beat ventricular time-volume relationships. Besides poor signal-to-noise ratio, calculating ventricular ejection fraction (EF) from the magnitude of the few well defined spikes is a simple, well known method.

Recently Iwata proposed a correction for the method and applied it to the right ventricle (*1*). Iwata's correction contains a principal error, but it is potentially useful if used as modified below.

Denote by V(ed) the ventricular volume at end-diastolic time (ed) and by V(es) its volume at the time of the succeeding end systole (es). By definition it stands:

$$EF = 1 - V(es)/V(ed). \quad (1)$$

If A(t) is the ventricular count rate and if V(t)~A(t), Equation 1 then reads:

$$EF = 1 - A(es)/A(ed.) \quad (2)$$

Equation 2 is used in standard method. Iwata, however, introduced ventricular indicator concentration, c(t), and proposed the following correction of Equation 2:

$$EF = 1 - (c(ed)/c(es)) \cdot A(es)/A(ed). \quad (3)$$

The apparent idea was that proportionally V(t) ~A(t) holds if indicator concentration does not change in the time from ED to ES. On the contrary, the ratio c(ed)/c(es), depicted from the low-frequency first-pass curve, can be used to correct for it. However, here and in Iwata's article the time from arbitrary diastole, ED, to the succeeding systole, ES, is the ejection phase, when all inputs to the ventricle are suspended and, assuming perfect mixing, the indicator concentration does not change. Consequently, as long as one analyzes only the ejection phases (descending limbs of the spikes), the equality, c(es) = c(ed), renders Equations 2 and 3 equivalent, which obviates the need for the correction of the standard method. In order to make more efficient use of these data, one can also analyze the filling phases (ascending limbs of the spikes), when es is arbitrary end-systole and ed is the succeeding diastole. Then