Localization of Fc and Fab Fragments of Nonspecific Polyclonal IgG at Focal Sites of Inflammation

Alan J. Fischman, Robert H. Rubin, Jennifer A. White, Elizabeth Locke, Robert A. Wilkinson, Mark Nedelman, Ronald J. Callahan, Ban An Khaw, and H. William Strauss

Division of Nuclear Medicine, Department of Radiology, and Division of Infectious Diseases, Massachusetts General Hospital; and the Departments of Radiology and Medicine, Harvard Medical School, Boston, Massachusetts

Intact IgG, Fc, Fab, and 1/2Fc (reduced and alkylated Fc) were coupled to DTPA, labeled with indium-111 and administered to rats to compare the ability of fragments of IgG to localize at focal sites of inflammation. Two sets of experiments were performed: First, 1, 6, 24, and 48 hr after injection, biodistribution was determined in healthy animals; second, localization at sites of inflammation was determined by scintillation camera imaging of animals with deep-thigh infection due to *Escherichia coli*. The biodistribution studies demonstrated differences in kidney and liver localization: IgG < Fc < Fab < 1/2Fc (kidney), Fc < 1/2Fc < IgG < Fab (liver). The imaging studies revealed that target-to-background ratio (T/B) and percent residual activity (%RA) for IgG was significantly greater ($p < 0.01$) than 1/2 Fc or Fab, and T/B for IgG was greater ($p < 0.01$) than Fc. These studies suggest that the Fc portion of IgG is the fragment with the best imaging properties.

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Three radiopharmaceuticals have been described for the detection of focal inflammation: gallium-67- (^{67}Ga) citrate (*1*), indium-111-labeled white blood cells (^{111}In-WBCs) (*2*), and recently ^{111}In-labeled nonspecific polyclonal IgG (^{111}In-IgG) (*3–5*). Understanding the mechanisms by which the different infection imaging agents localize in lesions may be important to identifying circumstances where one reagent may be preferable to the others. Acute infections, for example, appear to be imaged well with ^{111}In-IgG (*3,4*) and ^{111}In-WBCs (*6*), while chronic infection seems to be readily detected with ^{67}Ga (*6*).

Gallium-67, ^{111}In-WBCs, and ^{111}In-IgG concentrate in areas of inflammation by different mechanisms. Localization of ^{67}Ga has been attributed to: direct binding to bacteria (*7*); binding to components of white cells (*7*); and protein leakage at the site of inflammation carrying ^{67}Ga-transferrin into the lesion (*8*). Indium-111 WBCs localize by direct chemotaxis (*9*), causing a fraction of the labeled cells to find their way to the site of inflammation. In the case of ^{111}In-IgG, the mechanism of localization at sites of inflammation is less well defined.

IgG localizes inflammation due to a wide range of bacterial and fungal etiologies, and even inflammation due to chemical stimuli (*10*). This broad responsiveness makes it unlikely that the specific, antigen recognition, portion of the molecule is responsible for localization.

Infection and chemical irritant stimuli lead to a stereotyped response in the host, manifest by increased blood flow and tissue permeability, and a cellular infiltrate, consisting of granulocytes, macrophages, and lymphocytes. Since all of these cell types express Fc receptors on their surface, it has been postulated that IgG localizes at inflammatory sites by binding of the Fc portion of the molecule to receptors on inflammatory cells (a non-antigen specific mechanism) (*11*).

Recently, we demonstrated by autoradiography that the majority of ^{111}In-IgG in inflammatory lesions is confined to the extracellular space with minimal association to inflammatory cells (*12*). This finding, makes the Fc receptor hypothesis less likely and suggests that increased tissue permeability may make a major contribution to IgG localization.

To further evaluate the mechanism of IgG accumulation at focal sites of inflammation, we compared the localization of ^{111}In-labeled intact nonspecific IgG (containing both the antigen recognition Fab portion and the Fc portion) with multiple ^{111}In-labeled antibody fragments prepared by digesting the IgG with papain. The fragments tested were Fab, Fc, and 1/2Fc (reduced alkylated Fc) (Fig. 1).

MATERIALS AND METHODS

Preparations of Fc, Fab, and 1/2Fc Fragments

Human, nonspecific polyclonal IgG (Sandoglobulin®, San-

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For reprints contact: Alan J. Fischman, MD, PhD, Division of Nuclear Medicine, Massachusetts General Hospital, Fruit Street, Boston, MA 02114.

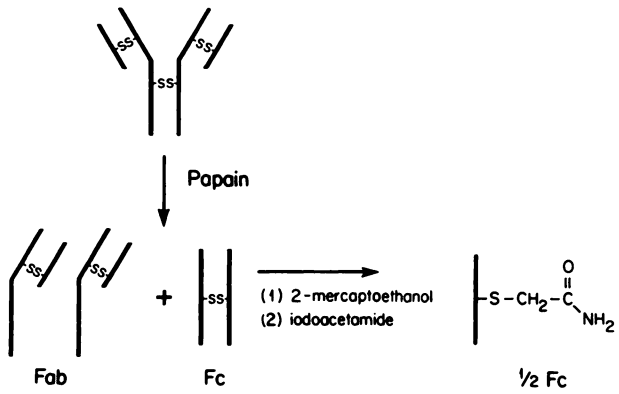

FIGURE 1
Diagram of the intact antibody and the papain digestion products Fc, Fab, and 1/2Fc. The structures illustrated here correspond to a generalized antibody molecule. In the various subclasses of human polyclonal IgG, additional interchain disulfide bonds are present.

doz, Inc., East Hanover, NJ) was used to prepare Fc, Fab, and 1/2Fc fragments. IgG (250 mg) was incubated with 2.5 mg of papain (Sigma, St. Louis, MO) for 1.5 hr at 37°C. The reaction mixture was applied to a calibrated column of Sephadex G-100 and the fractions eluting in the 50-kDa range (Fc plus Fab) were pooled. The Fc and Fab fragments were separated on a protein A Sepharose column. The fractions containing the Fc and Fab fragment were individually pooled and further purified by DEAE cellulose chromatography. Purified Fc fragments were reduced with 2-mercaptoethanol and alkylated with iodoacetamide to produce 1/2Fc. All products were >90% pure by immunoelectrophoresis, polyacrylamide gel electrophoresis (PAGE), high-performance liquid chromatography (HPLC), and Ouchterlony immunodiffusion (*13*).

Indium-111 Labeling

Intact IgG, Fc, Fab, and 1/2Fc were radiolabeled with ^{111}In (Indium Chloride, Amersham, Arlington Heights, IL) via the diethylenetriaminepentaacetic acid (DTPA) carboxycarbonic anhydride chelate method (*13,14*). Briefly, isobutylchloroformate (0.25 mmoles) was added to a cooled solution (ice bath) of the triethylamine salt of DTPA (0.25 mmoles in 2 ml of acetonitrile) and the mixture was stirred for 30 min. An aliquot of the resulting solution of the mixed carboxycarbonic anhydride of DTPA was then added to a cooled solution of intact antibody, Fab, Fc, or 1/2Fc dissolved in 0.1 *M* $NaHCO_3$ with the pH maintained at between 7 and 8 by addition of 0.1 *M* $NaHCO_3$. The molar ratio of mixed carboxycarbonic anhydride to protein fragment was 100:1 (resulting in 4–6 DTPA groups/protein molecule). The mixture was dialyzed against 0.1 *M* acetate buffer pH 5.0 at 4°C overnight and applied to a column of Sephadex G-25. The protein fraction, eluting at the void volume of the column, was dialyzed against normal saline. A 0.5-ml aliquot of the resulting DTPA conjugate (containing ~ 0.5 mg of protein) was diluted with 0.5 ml of 1.0 *M* citrate buffer (pH 5.0) and combined with 1–2 mCi of $^{111}InCl_3$ immediately prior to use. The mixture was incubated for 30 min at room temperature, applied to a column of Sephadex G-25, and the protein fraction isolated. The pooled protein fractions were passed through a 0.22-micron filter (Millipore Corp., Bedford, MA) prior to injection. Chemical purity of the labeled products was determined to be >90% by HPLC and PAGE.

Animal Model

A single clinical isolate of *Escherichia coli* was employed to produce focal infection. The bacteria were incubated overnight on trypticase soy agar plates at 37°C, and individual colonies were diluted with sterile normal saline to produce a turbid suspension containing ~ 2×10^9 organisms/ml. Male Sprague-Dawley rats, weighing ~200 g (Charles River Breeding Laboratories, Burlington, MA), were anesthetized with ketamine and their left posterior thigh traumatized by pinching with a hemostat. Then 0.1 ml of a suspension containing ~2×10^8 organisms was injected into the traumatized thigh muscle (*10*).

Imaging

Twenty-four hours after bacterial innoculation, when gross swelling was apparent in the thigh, one of the radiolabeled proteins (~0.25 mg/kg labeled with 100–150 μCi of ^{111}In for each preparation) was injected intravenously via the tail vein. Following injection of the radiolabeled reagent, serial anterior whole-body scintigrams of ketamine anesthetized animals were acquired using a standard field of view scintillation camera equipped with a parallel-hole medium-energy collimator interfaced to a dedicated computer system (Technicare 100, Technicare 450, Solon, Ohio). Images were recorded for a preset time of 10 min/view with a window centered to include both the 173 and 247 keV photopeaks of ^{111}In.

Data Analysis

Regions of interest were drawn around the infected area, a similar area in the contralateral thigh, and the whole animal to calculate target-to-background ratio (T/B) (infected muscle/contralateral thigh) and percent residual activity (%RA) (infected muscle/whole animal). At the conclusion of imaging, selected animals were killed with ether anesthesia, autopsied, and the infections were verified by culture.

Biodistribution

Groups of 24 uninfected rats were injected with 15 μCi of the selected reagent labeled with ^{111}In to determine the biodistribution of IgG, Fc, 1/2Fc, or Fab at 1, 6, 24, and 48 hr (each reagent was evaluated in six animals at each time point). Samples of blood, lung, liver, spleen, kidney, muscle, and intestine were weighed and radioactivity was measured in a well-type gamma counter (LKB model #1282, Wallac Oy, Finland). In rats receiving IgG, the blood was centrifuged to determine the relative fraction of activity that was cell associated. To correct for radioactive decay and permit calculation of activity in each organ as a fraction of the administered dose, aliquots of the injected doses were counted simultaneously. The results were expressed as percent injected dose per gram (%ID/g) and percent injected dose/organ. For blood and muscle, %ID was calculated as previously described (*15*).

Biodistribution was determined in uninfected rats for two reasons:

1. Previous results from this laboratory have demonstrated that only in cases of severe sepsis causing hypotension is the distribution of cardiac output affected by infection (*16*).
2. In our human imaging studies with ^{111}In-IgG, in over

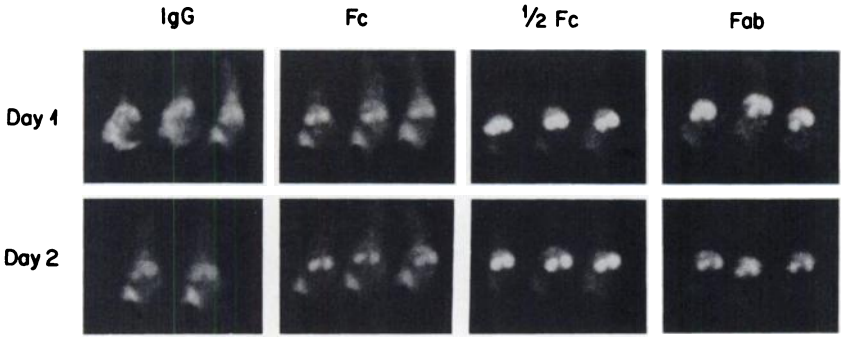

FIGURE 2
Gamma camera images (anterior) of groups of rats with *E. coli* deep-thigh infections, 1 and 2 days after injection of ^{111}In-labeled intact IgG, Fc, 1/2Fc, or Fab. Infections were established 24 hr prior to injection. These images represent a subs-et of the data presented in Figure 3. Proceeding from the top of each panel, the areas of increased concentration of radioactivity are as follows: cardiac blood pool, liver, kidneys, and area of infection.

150 patients, no effect of infection on biodistribution was detected (unpublished results).

Statistical Methods

The results of the imaging and biodistribution studies were evaluated by analysis of variance followed by Duncan's new multiple range test (*17*). All results are expressed as mean ± s.e.m.

RESULTS

Imaging

The animals tolerated the intravenous administration of IgG, Fc, 1/2Fc, and Fab without apparent ill effects. IgG and Fc had definite localization at the site of infection at both 24 and 48 hr postinjection. The images acquired with radiolabeled 1/2Fc and Fab consistently showed lower T/B ratio and %RA, but the 1/2Fc images tended to be slightly better (Figs. 2 and 3).

Analysis of variance of the T/B data showed a significant main effect of protein preparation: $F_{3,57} = 45.80$, $p < 0.0001$; a significant main effect of time: $F_{1,57} = 14.06$, $p < 0.0005$; and a significant preparation by time interaction: $F_{3,57} = 5.38$, $p < 0.005$. At both 24 and 48 hr after injection, the T/B ratio for intact IgG was significantly higher ($p < 0.01$) than for Fc, 1/2Fc, or Fab. At 48 hr, the T/B ratios for Fc and 1/2Fc were significantly greater ($p < 0.05$) than for Fab. For %RA, analysis of variance showed a significant main effect of protein preparation, $F_{3,57} = 15.88$, $p < 0.0001$; however the effects of time and the preparation by time interaction were not significant ($p > 0.35$ and $p > 0.97$). At both times, the %RA for intact IgG was significantly greater ($p < 0.01$) than for 1/2Fc or Fab. At 24 hr, the %RA for Fc was significantly greater ($p < 0.01$) than 1/2Fc or Fab.

Biodistribution

All four antibody preparations showed a monotonic decrease in blood concentration over time (Fig. 4). At all four time points after injection, the amount of IgG in the circulation was significantly greater than the other three preparations ($p < 0.01$), and the amount of Fc was significantly greater than 1/2Fc and Fab ($p < 0.01$). At 6 hr, the amount of 1/2Fc was greater than Fab ($p < 0.01$). The calculated blood clearance half-times ($t_{1/2}$) in hours were: IgG, 36.4; Fc, 32.5; 1/2Fc, 22.3; and Fab, 12.8. Less than 1% of the circulating IgG activity was cell associated.

The biodistribution of the four antibody preparations is shown in Figure 5. Detailed statistical analysis of the

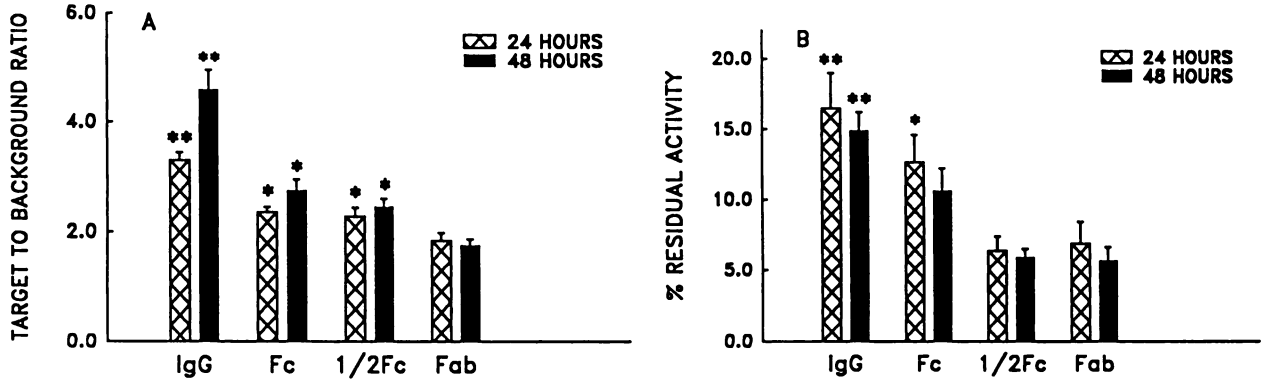

FIGURE 3
Target-to-background ratio (count density in infected muscle/count density in contralateral thigh) and percent residual activity (radioactivity in infected muscle/radioactivity in whole animal) of *E. coli* deep-thigh infections in rats. The animals were imaged 1 and 2 days after injection of ^{111}In-labeled intact IgG, Fc, 1/2Fc, or Fab. (A) Target-to-background ratio, $^{**}p < 0.01$ (IgG compared to Fc, 1/2Fc, or Fab at both time points), $^{*}p < 0.05$ (Fc and 1/2Fc, compared to Fab at both time points). (B) Percent residual activity, $^{**}p < 0.01$ (IgG compared to Fc, Fab, and 1/2Fc at both time points), $^{*}p < 0.01$ (Fc compared to 1/2Fc at 24 hr). In all cases, infections were established 24 hr prior to injection. Each point is the mean ± s.e.m. for 6 to 10 animals.

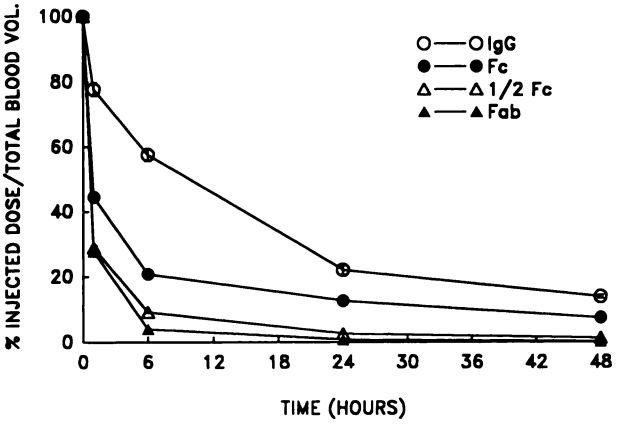

FIGURE 4
Blood clearance curves (% total initial radioactivity activity injected) for ^{111}In-labeled intact IgG, Fc, 1/2Fc, or Fab in uninfected rats. Each point is the mean ± s.e.m. for six animals.

data is presented in the Appendix. The pattern of pulmonary accumulation of all the antibody preparations was similar to the pattern in blood. In liver, Fc accumulated to the least extent compared to the other preparations. Intact IgG showed progressive accumulation in spleen. However, in general, splenic accumulation of the other antibody preparation decreased with time. At 24 and 48 hr postinjection, Fc and 1/2Fc had the lowest levels of accumulation in spleen. Renal accumulation was greatest for 1/2Fc at all time points and only minor differences in accumulation of the other preparations were detected. In muscle, accumulation of the different antibody preparations was similar except at 24 and 48 hr postinjection when accumulation of IgG was slightly lower than Fc and 1/2Fc and Fab were significantly lower than Fc and IgG. In the GI tract, Fab accumulation was highest and 1/2Fc accumulation was lowest at all time points. Accumulation of intact IgG and Fc started at a similar level to Fab, but decreased in parallel to blood activity.

DISCUSSION

Radiolabeled human polyclonal IgG readily accumulates in inflammatory lesions to an extent sufficient to yield excellent external images (*3,5*). The results of this report establish that Fc fragments of IgG have very similar imaging properties to intact IgG. In contrast, Fab and 1/2Fc fragments are relatively poor infection imaging agents.

There are several possible explanations for the different imaging properties of the various IgG fragments.

Fc Receptor Hypothesis: The binding characteristics of IgG to inflammatory cells are consistent with an Fc receptor-mediated mechanism of IgG localization. Pooled human IgG_1 binds to peripheral blood monocytes with high affinity ($K_a = \sim 5 \times 10^{-8}\ M^{-1}$) and there are 20,000–30,000 sites per cell (*18*). Also, while this antibody preparation binds to polymorphonuclear leukocytes with lower affinity ($K_a = \sim 10^{-6}\ M^{-1}$) a high level of total binding can still occur since there are significantly more binding sites per cell (~100,000) (*18*). These binding characteristics compare favorably with other receptor systems, i.e., transferrin receptors on reticulocytes ($K_a = \sim 10^{-7}\ M^{-1}$; $\sim 10^5$ sites/cell) (*19*).

Despite these facts, the Fc receptor hypothesis remains problematic for several reasons:

1. In a previous study using the same infection model as employed here, we were unable to demonstrate significant association of IgG with inflammatory cells (*12*).
2. The physiologic, circulating levels of IgG (8–15 grams/liter serum) should block uptake.
3. Despite the fact that different subclasses of IgG have marked differences in Fc receptor binding,

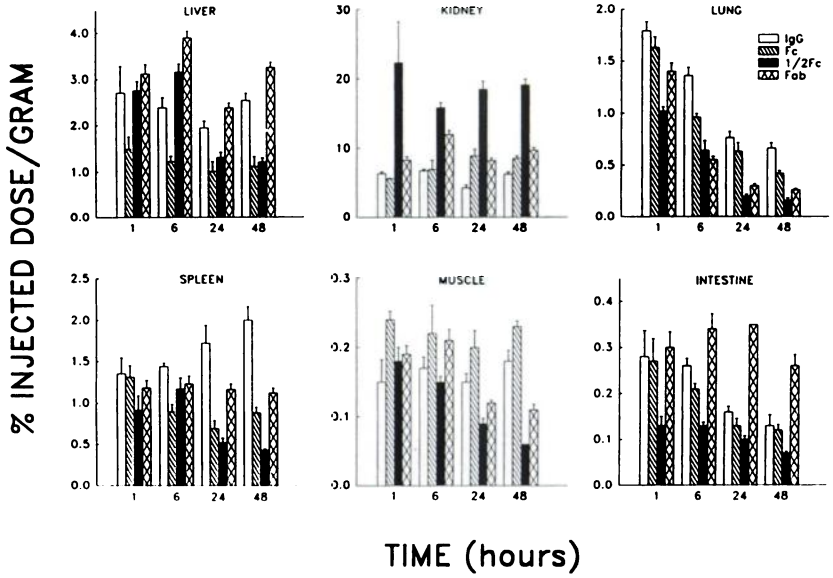

FIGURE 5
Biodistribution of ^{111}In-labeled intact IgG, Fc, 1/2Fc, or Fab in uninfected animals expressed as %ID/g. Each point is the mean ± s.e.m. for six animals. Detailed statistical analysis of the data is presented in the Appendix.

previous studies in animals have demonstrated that all subclasses of human IgG have remarkably similar inflammation imaging properties (unpublished results).

Thus, it appears that localization of IgG at regions of inflammation is probably not Fc receptor-mediated. However, the fact that Fc fragments localize at sites of infection better than Fab fragments suggests that Fc fragments may have physical chemical properties that favor localization.

Differential Clearance Rate. Fc fragments have only slightly faster blood clearance than intact IgG, however, both these proteins have prolonged circulating half-times relative to Fab and 1/2Fc (Fig. 4). Therefore, it is possible that the superior imaging properties of IgG and Fc are due to greater time of exposure of the lesions to the radiolabeled proteins. This factor could explain greater total lesion accumulation of IgG or Fc, but offers no explanation for differences in T/B ratio or %RA, since the background is exposed to the circulating pool of radiolabeled protein for the same time as the lesion. Since, the background tissue for the experimental infections is normal muscle, some insight into the difference in T/B ratio and %RA between the various protein preparations comes from the biodistribution data. At early times (1 and 6 hr), %ID/g normal muscle was only slightly different for the four preparations, and for intact IgG and Fc there was minimal change at the later times (24 and 48 hr). With Fab and 1/2Fc, the amount of activity in muscle decreases at the later times and was significantly less than IgG or Fc. This suggests that the superior images recorded with IgG and Fc compared to Fab and 1/2Fc cannot be explained by lower accumulation of IgG and Fc in normal muscle. However, the fact that at 24 and 48 hr postinjection radioactivity accumulation in normal muscle was slightly greater for Fc than IgG, may partially explain the slightly better T/B ratio and %RA of IgG compared to Fc. Similarly, greater accumulation of Fab in normal muscle compared to 1/2Fc may explain the slightly higher values of these parameters for 1/2Fc compared to Fab.

It has been reported that accumulation of IgG in tumors can be explained in terms of a difference in influx and efflux rate constants (*20*). Recently, we determined that the influx rate constant for IgG is elevated much more than the efflux rate constant in infected muscle (unpublished results). If as expected, this difference is less pronounced for the smaller antibody fragments, the superior imaging properties of intact IgG could be explained. However, since Fc and Fab fragments are of similar size, superiority of Fc cannot be explained.

While it does not appear that Fc offers any general advantage over intact IgG for imaging focal sites of inflammation, it could be useful in specific instances. Based on our clinical experience with ^{111}In-IgG for imaging focal sites of inflammation in humans, it has become clear that the high level of accumulation of intact IgG in liver and spleen, makes the right and left upper quadrants of the abdomen extremely difficult to evaluate (*3,5*). The fact that Fc has ~50% lower accumulation in liver and spleen and similar accumulation in bowel compared to IgG (Fig. 5) suggests that ^{111}In-Fc might be superior to intact IgG for detecting perihepatic and splenic lesions. Similarly, the observation that Fc clears from the blood pool faster than IgG (Fig. 4) suggests a possible role for this agent in imaging vascular infections, where differentiation of lesion accumulation of radiolabel from residual blood-pool activity is critical (*21*).

Although the inflammation imaging properties of the 1/2Fc fragment of IgG were inferior to IgG or Fc, the fact that it was slightly better than Fab is interesting and warrants further investigation. It is possible that other Fc fragments, such as mFc, sFc, or stFc, (*22*) might be more effective imaging agents.

APPENDIX

Statistical analysis of biodistribution data is presented below.

Blood

Analysis of variance demonstrated a significant main effect of time: $F_{3,94} = 1000.0$, $p < 0.0001$; a significant main effect of preparation: $F_{3,94} = 978.5$, $p < 0.0001$; and a significant preparation by time interaction: $F_{9,94} = 85.3$, $p < 0.0001$. At all four time points after injection: the amount of IgG in the circulation was significantly greater than the other three preparations ($p < 0.01$) and the amount of Fc was significantly greater than 1/2Fc and Fab ($p < 0.01$). At 6 hr, the amount of 1/2Fc was greater than Fab ($p < 0.01$). For IgG and Fc, the amount of residual blood-pool activity was significantly lower ($p < 0.01$) at each successive time point. A similar pattern was seen for 1/2Fc and Fab, except the decreases between 24 and 48 hr were not statistically significant.

Lung

The pattern of accumulation in lung of all the antibody preparations was similar to that seen in blood (Fig. 5). Analysis of variance showed a significant main effect of time: $F_{3,94} = 109.4$, $p < 0.0001$; a significant main effect of preparation: $F_{3,94} = 117.0$, $p < 0.0001$; and a significant preparation by time interaction: $F_{9,94} = 2.54$; $p < 0.05$. Regression analysis showed a highly significant correlation between lung and blood radioactivity; $r = 0.94$ ($p < 0.001$). At all four time points after injection, the amount of IgG in lung was significantly greater than Fc, 1/2Fc, or Fab. At 1 hr, the amount of Fc and Fab were greater than 1/2Fc ($p < 0.01$). At 6 and 24 hr, the amount of Fc was greater than 1/2Fc and Fab ($p < 0.01$). Over the first 24 hr, the amount of IgG in lung was significantly lower at each successive time point ($p < 0.01$). The amount at 48 hr was not significantly different from 24 hr. For Fc, the amount of activity in lung at 1 hr was greater than that at 6 hr ($p < 0.05$), 24 hr ($p < 0.01$), and 48 hr ($p <$

0.01), and the amount at 6 hr was greater than that at 24 and 48 hr ($p < 0.01$). A similar pattern was observed for 1/2Fc and Fab.

Liver

Analysis of variance showed a significant main effect of time: $F_{3,93} = 12.5$, $p < 0.0001$; a significant main effect of preparation: $F_{3,93} = 228.8$, $p < 0.0001$; and a significant preparation by time interaction: $F_{9,93} = 3.68$, $p < 0.001$. At 1 hr, the amount of Fab, IgG, and 1/2Fab was greater than Fc ($p < 0.01$). At 6 hr, the amount of Fab, IgG, and 1/2Fc was greater than IgG and Fc ($p < 0.01$). At 24 hr, the amount of Fab was greater than Fc and 1/2Fc ($p < 0.01$). At 48 hr, the amount of IgG and Fab was greater than Fc and 1/2Fc ($p < 0.01$). Over the course of 48 hr, there was no significant change in the amount of IgG in the liver. For Fc, the amount of activity in the liver at 1 hr was greater than at 48 hr ($p < 0.05$). For 1/2Fc, greater accumulation of activity was seen in the liver at 6 hr compared to all other times. Also, the amount of 1/2Fc on liver at 1 hr was greater than at 24 hr or 48 hr ($p < 0.05$). For Fab, the amount of activity in liver was greater at 1, 6, and 48 hr than at 24 hr ($p < 0.05$).

Spleen

Analysis of variance showed a significant main effect of preparation: $F_{3,94} = 93.0$, $p < 0.0001$, and a significant preparation by time interaction: $F_{9,94} = 8.3$, $p < 0.0001$. At all four time points, the amount of IgG in spleen was significantly greater than Fc, 1/2Fc, or Fab ($p < 0.01$). At 1 hr the amount of Fab was greater than 1/2Fc ($p < 0.01$). At 24 hr, the amount of Fab was greater than 1/2Fc or Fc ($p < 0.01$). At 48 hr after injection, the amount of IgG in spleen was greater than at 1 hr ($p < 0.01$), 6 hr ($p < 0.01$) and 24 hr ($p < 0.05$). For Fc, the amount of activity in spleen was greater at 1 hr than at 24 hr ($p < 0.01$) or at 48 hr ($p < 0.05$). For 1/2Fc, the amount of activity in spleen was greater at 6 hr than at 1 hr ($p < 0.05$), 24 hr ($p < 0.01$), or 48 hr ($p < 0.01$). A significant effect of time on Fab accumulation in spleen was not detected.

Kidney

Analysis of variance showed a significant main effect of time: $F_{3,94} = 14.7$, $p < 0.0001$; a significant main effect of preparation: $F_{3,94} = 365.8$, $p < 0.001$; and a significant preparation by time interaction: $F_{9,94} = 9.2$, $p < 0.0001$. At all four time points, the amount of 1/2Fc in kidney was greater than IgG, Fc and Fab ($p < 0.01$). At 1 hr, the amount of Fab in kidney was greater than Fc ($p < 0.01$) and IgG ($p < 0.05$). At 6 hr, the amount of IgG and Fab in kidney was greater than IgG and Fc ($p < 0.01$). At 24 hr, the amount of Fc in kidney was greater than IgG ($p < 0.01$). At 6 hr and 48 hr after injection, the amount of IgG in kidney was greater than at 24 hr ($p < 0.05$). For Fc, the amount of activity in kidney at 24 hr and 48 hr was greater than at 1 hr and 6 hr ($p < 0.01$). For 1/2Fc, the amount of activity in kidney was greater at 1, 24, and 48 hr than at 6 hr ($p < 0.01$). For Fab, the amount of activity in kidney was greater at 6 hr than at 1 hr ($p < 0.01$) or 24 hr ($p < 0.05$).

Muscle

Analysis of variance showed a significant main effect of time: $F_{3,93} = 3.30$, $p < 0.05$; a significant main effect of preparation: $F_{3,93} = 52.3$, $p < 0.0001$; and a significant preparation by time interaction: $F_{9,93} = 3.27$, $p < 0.005$. At 1 hr after injection, the amount of Fc in muscle was greater than 1/2Fc ($p < 0.05$). At 6 hr, the amount of IgG, Fab, and Fc was greater than 1/2Fc ($p < 0.01$). At 24 hr and 48 hr, the amount of Fc in muscle was greater than all the other preparations ($p < 0.01$); and the amount of IgG was greater than 1/2Fc and Fab ($p < 0.01$). For IgG, the amount of activity in muscle was greater at 48 hr ($p < 0.01$) than at 1 hr. For Fc, the effect of time on accumulation in muscle was not significant. For 1/2Fc, the amount of activity in muscle at 1 hr was greater than at 24 hr ($p < 0.05$) and 48 hr ($p < 0.01$); and the amount of activity at 6 hr was greater than at 48 hr ($p < 0.01$). For Fab, the level of accumulation was greater at 1 hr and 6 hr than at 24 and 48 hr ($p < 0.05$).

GI Tract

Analysis of variance showed a significant main effect of time: $F_{3,93} = 27.4$, $p < 0.0001$; a significant main effect of preparation: $F_{3,93} = 227.9$, $p < 0.0001$; and a significant preparation by time interaction: $F_{9,93} = 4.30$, $p < 0.005$. At 6, 24, and 48 hr after injection, the amount of Fab in the GI tract was greater than Fc and 1/2Fc ($p < 0.01$); and the amount of Fc was greater than 1/2Fc, 1 hr and 6 hr ($p < 0.01$), 48 hr ($p < 0.05$). At 1 hr and 6 hr, the amount of IgG in the GI tract was greater than at 24 hr and 48 hr ($p < 0.01$). For Fc, the amount of activity in the GI tract at 1 and 6 hr was greater than at 24 hr and 48 hr ($p < 0.01$). For 1/2Fc, a significant effect of time on GI level of activity was not detected. For Fab, the amount of activity in the GI tract at 6 and 24 hr was greater than at 48 hr.

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