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The biological half-lives of four rat immunoglobulin isotypes

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Summary. Purified preparations of monoclonal rat IgG2a, IgG1, IgM and monomeric IgA, and of polyclonal IgG, were lightly radiolabelled with ^{125}I and injected intravenously into rats. Blood samples were taken so that the half-life of each immunoglobulin isotype could be calculated. Half-life was defined as the time taken for the specific radioactivity present in the blood 24 h after injection to fall by half. The half-life values thus obtained were 106 h for IgG2a, 53 h for IgG1, 63 h for polyclonal IgG, 25 h for IgM and 27 h for monomeric IgA.

INTRODUCTION

Antibodies of the same specificity within a given species possess different biological properties which depend on the immunoglobulin class or subclass (isotype) to which they belong. These biological properties include the ability to fix complement, to be transported across epithelia, to bind to various cells, to sensitize for anaphylaxis and also the length of time for which a particular isotype remains in the circulation (e.g. Spiegelberg, 1974).

Measurements of such biological half-lives can be made only in species in which the Ig isotypes can be identified, purified in amounts sufficient for passive transfer and their fate in the circulation of the recipient monitored in blood samples taken at appropriate intervals. The Igs of rats comprise IgM, IgA, IgE, IgG1, IgG2a, IgG2b, IgG2c, (Bazin, Beckers & Querinjean, 1974) and IgD (Bazin, Beckers, Urbain-Vansanten, Pauwels, Bruyns, Tilkin, Platteau & Urbain, 1978) and for most of these, transplantable immunocytomas are available. Reliable estimates for the half-lives both in the circulation and the skin have been reported only for IgE and IgG2a (Tada,Okumura, Platteau, Beckers & Bazin, 1975). In the case of polymeric IgA its active removal from the blood by the liver (Orlans, Peppard, Reynolds & Hall, 1978) is too rapid for any evaluation of catabolism.

In this study we have compared the rates at which purified monoclonal IgM, IgG1, IgG2a and monomeric IgA as well as polyclonal IgG are cleared from the blood of normal rats after intravenous injection.

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MATERIALS AND METHODS

Animals and experimental procedures

Paraproteins were isolated from the sera of rats of the Lou/Wsl strain bearing the following Ig secreting plasmacytomas subcutaneously: IR31 (IgG1), IR33 (IgG2a) IR202 (IgM) and IR473 (IgM). The IgA-forming tumour IR461 was grown as ascites, and the IgA prepared from ascitic fluid.

The clearance of the purified radiolabelled proteins was measured in 12 week old male Wistar rats weighing 200–250 g. They were kept in modified Bollman restraining cages for the duration of the experiments.

Blood samples of about 0·1 g were taken from the tail; repeated bleeding, after a single small cut, was possible over several days if the end of the tail was kept bandaged. To facilitate injection of the radiolabelled material a nylon cannula was inserted into the femoral vein under light ether anaesthesia, at least 18 h before injection.

Radiolabelling of proteins and estimations of radioactivity

Proteins were trace-labelled with ^{125}I by the chloramine-T method (Greenwood & Hunter, 1963) to a final activity of 0·03–0·05 μCi/μg. Radioactivity was measured in a Packard Auto Gamma Scintillation Spectrometer S260. The radioactive blood samples of about 0·1 g were collected into weighed tubes, reweighed, counted and the radioactivity expressed as c.p.m./g of whole blood.

Purified immunoglobulins

IgA. The isolation and properties of monomeric IgA from the IR461 ascites by two different methods, one involving gel filtration and ion-exchange chromatography and the other affinity chromatography, have been described (Peppard, 1979) as has that of polymeric IgA from the same source (Orlans *et al*, 1978).

IgM. The precipitates formed after thorough dialysis against distilled water of sera containing the IR202 and IR473 paraproteins were collected by centrifugation, dissolved in phosphate buffer and fractionated on columns of Ultrogel AcA22 and Sephadex G-200 respectively. The IgM-containing exclusion peaks from each column were collected. The IR473 material was recycled on G-200, and a portion of the IgM peak specifically purified on a column of specifically purified rabbit antibodies to rat μ chains coupled to CNBr Sepharose; the bound protein was eluted with 3 M NH_4SCN and dialyzed extensively before use. The IR202 protein was re-precipitated by dialysis against phosphate buffer (0·025 M, pH 7·0) and then fractionated twice on the Ultrogel AcA22 column.

Monoclonal IgG1 and IgG2a. These were made by stepwise elution from a column (2·6 × 100 cm) of DEAE cellulose using the following phosphate buffers sequentially: (a) 0·01 M, pH 7·4; (b) 0·03 M, pH 6·0; (c) 0·05 M, pH 5·3; (d) c + 0·05 M NaCl; (e) c + 0·1 M NaCl. The IgG2a (IR33) protein eluted mainly with buffer a and IgG1 (IR31) with buffer e. The polyclonal IgG preparation consisted of the pooled eluates obtained with buffers a, b and c.

All the purified protein preparations were examined by immunoelectrophoresis with various class- and subclass-specific antisera and with an antiserum to whole rat serum; electrophoresis in agarose gels was also used.

RESULTS

The homogeneity of the purified Ig preparations varied. The IgG2a contained readily detectable IgG2b amounting to about 20% of the total protein; the IgG1 was free of IgG2 but contained a very small amount of a non-Ig protein. One of the IgM preparations from IR473 contained α2 macroglobulin but this was not present in the other prepared by affinity chromatography. No contaminants were detected in the monomeric IgA preparations or in the IR202 IgM.

The rate of the fall of radioactivity in the blood of rats injected with various ^{125}I-Ig preparations is shown in Fig. 1. The half-lives shown in Table 1 were obtained by plotting the same results on semi-log paper and calculating the time taken for the radioactivity found in blood samples 24 h after injection to fall to half of this value. This method was chosen because, with one exception (monomeric IgA), none of the decay curves became strictly linear. The 24 h period after injection was considered adequate for the clearance of any damaged protein or unbound ^{125}I and for equilibration between the intra- and extravascular compartments. Also, by using this particular time span it was possible not to use values below 10% of the injected dose where the effects of any contaminating radiolabelled protein would be most marked.

Table 1. Half-lives of rat immunoglobulin isotypes

Isotype	Source	Half-life (h ± SE)
IgM	IR202, IR473	24·8 ± 1·2 (8)
Monomeric IgA	IR461	27·2 ± 1·9 (5)
IgG1	IR31	53·1 ± 7·9 (4)
IgG2a	IR33	106·0 ± 14·3 (3)
IgG	Normal serum	63·0 ± 7·0 (2)

Half-life was calculated as the time taken for the specific radioactivity present in the blood 24 h after injection to fall by half. The number of rats used in each case to calculate the half-life values is shown in parentheses.

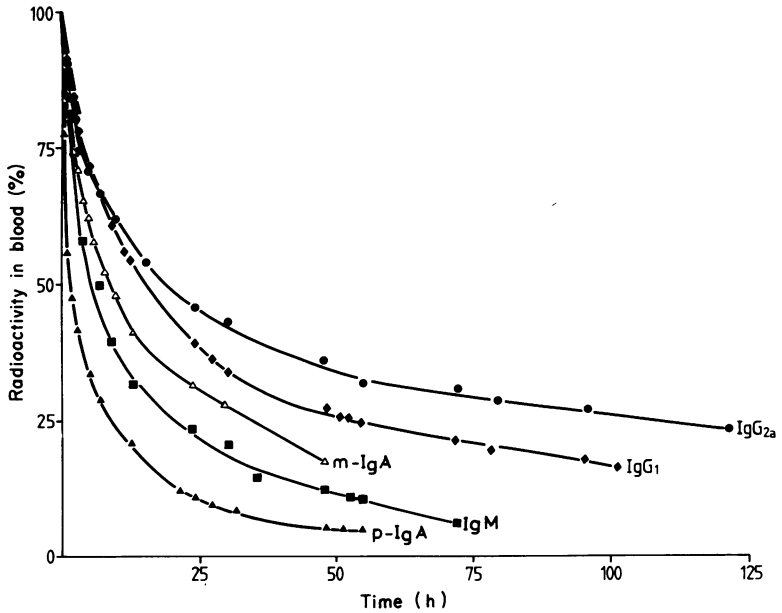

Figure 1. The clearance of radiolabelled immunoglobulins from the bloodstream of rats. The radioactivity present in the blood at each time point is plotted as percentage of that present at 15 min after injection. Each point is derived from the number of rats shown in Table 1. Those shown for IgM are results obtained using two different myeloma proteins. The clearance of polymeric IgA (p-IgA) is shown for comparison.

DISCUSSION

The results clearly show substantial differences in the rates at which different Ig isotypes are cleared from the circulation of rats *in vivo*. The half-lives in Table 1 probably provide a valid comparison of the various proteins relative to one another as they were isolated and radiolabelled by similar, mild procedures. The absolute values for the half-lives *in vivo* of the native or biosynthetically labelled proteins may well be greater as iodination has been shown to decrease the values. Tada *et al.* (1975) obtained values of 5·0 days for the half-life of both mono- and polyclonal IgG2a, in fair agreement with our result of 4·4 days; the half-life of the same monoclonal IgG2a protein measured by quantification of the idiotypic determinant was 6·1 days.

The half-life of rat IgG1 has not been measured before and was found to be only half that of IgG2a. A similar ratio in the catabolic rates of IgG2a and IgG1 in mice was reported by Fahey & Sell (1965) but not by Bazin & Malet (1969) who, however, questioned the purity of their IgG subclass preparation.

The preparation of normal IgG contained some IgG1 but consisted mainly of IgG2. Its clearance rate was much higher than that of IgG2a and nearer that of IgG1, suggesting that in rats, as in mice, (Fahey & Sell, 1965) IgG2a has the longest life in the circulation. It is important to remember, however, that so far no structural homology has been demonstrated for IgG2a in rats and mice. Such homology does exist for the IgG1 subclass of the two species and has been detected by the cross-reactivity of some antisera specific for rat or mouse γ1 chains (unpublished results).

Unlike polymeric IgA which disappears from the circulation extremely rapidly (Orlans *et al.*, 1978) monomeric IgA was found to remain in the blood slightly longer than IgM, with a half-life of 27 h.

Previous estimates for the half-life of polyclonal IgM were 56–64 h (Van Breda Vriesman & Feldman, 1972; Cremer, Taylor, Lenette & Hagens, 1973), much longer than the value of 25 h found in this study with two different monoclonal IgM proteins. However, polyclonal rat IgM is notoriously difficult to purify, and most of the earlier results were derived from whole body counts. Our lower value is consistent with the values of 0·5 days for mouse IgM (Fahey & Sell, 1965; Bazin & Malet, 1969), and with the relative catabolic rates of IgM and IgG in man where IgM is catabolized four to five times faster than IgG (Spiegelberg, 1974).

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