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Research Article

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The hypercatabolism of IgG could not be correlated with age, weight, sex, duration of disease, joint erosions, corticosteroid therapy, erythrocyte sedimentation rate, rheumatoid factor titer, serum IgG concentration, or circulating immune complexes as measured by the Raji cell radioimmunoassay.

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INTRODUCTION

The pathogenesis of rheumatoid arthritis (RA)[1] remains undefined, but abnormalities of humoral immunity have been well appreciated in this disorder and include hypergammaglobulinemia, with particular elevation of the plasma IgG concentration (1), presence of anti-IgG antibodies (2), and immune complex formation (1, 3) with complement consumption (4). Because of these phenomena, RA patients may metabolize IgG quite differently than normal subjects.

During the past 20 yr, numerous studies (5–16) have assessed the metabolism of normal homologous IgG labeled with radioiodine in RA patients. In most instances, faster turnover was found in RA patients than in normal subjects, but some authors (8, 12) found no difference. Duration of the studies and methods of calculating fractional catabolic rates (FCR) varied in these reports; consequently, absolute values are difficult to compare. None of the prior studies attempted to distinguish between intravascular and extravascular catabolism, and they employed methods of kinetic analysis which assumed only the former.

Recent evidence (17, 18) has suggested that IgG from RA patients may vary structurally from normal IgG, and alterations in metabolism of RA IgG as compared to normal IgG have been described in mice (15).

[1] *Abbreviations used in this paper:* ANA, antinuclear antibodies; Auto, autologous; E, extravascular; ESR, erythrocyte sedimentation rate, Westergren; FCR, fractional catabolic rate (percent per day); Homo, homologous; HRA, highly hypercatabolic rheumatoid arthritis; IgG-RF, IgG rheumatoid factor; MRA, mildly hypercatabolic rheumatoid arthritis; P, plasma; RA, rheumatoid arthritis; RF, rheumatoid factor; U, urine.

Four studies (12–14, 16) have measured the metabolism of RA IgG in RA patients. In three of these studies, it was compared with normal IgG. No clear differences were found, but details of these studies generally were not given.

The increased FCRs found by previous workers did not correlate with laboratory indices of disease, such as rheumatoid factor (RF) titers or erythrocyte sedimentation rates (ESR) (5, 6, 10, 11). One of these groups (11) assessed clinical disease activity by the number of involved joints and reported a positive correlation with higher FCRs. There are no data in these studies to indicate whether correlations were sought between FCR and circulating immune complexes, articular erosions, or manifestations of extra-articular disease.

The aims of the current study were to: (*a*) reassess IgG metabolism in RA by a recently developed method of kinetic analysis capable of measuring catabolism both intravascularly and extravascularly; (*b*) compare the metabolism of autologous RA IgG and normal homologous IgG in RA by the above-noted method; and (*c*) check extensively for correlations between IgG metabolism and clinical and laboratory indices of RA.

METHODS

Subjects. Tables I and II list the characteristics of the subjects studied. 10 normal ambulatory subjects were included in the study and served as controls (two were studied twice) (Table I). Control subjects were predominantly female (mean age of 35 yr) with no physical, clinical, or laboratory evidence of RA. 18 patients with definite or classical seropositive RA, as defined by the American Rheumatism Association (19), were studied (two patients were studied twice) (Table II). All patients were hospitalized in the Clinical Research Center at Scripps Clinic and Research Foundation, La Jolla, Calif. for a period of 3 wk. Ages of the RA patients ranged from 30 to 79 yr with a mean of 60. Duration of arthritis ranged from 2 to 35 yr. All patients had RF titers of 320 or greater. No patient had gastrointestinal or renal disease. Anti-inflammatory and other routine medications were continued unchanged throughout the study. One patient (Pa. D.) was maintained on a previously established dose of 150 mg per day cyclophosphamide. Another (J. R.) was on cyclophosphamide in intravenous (i.v.) pulse doses (total of five, ranging from 400–1,000 mg) and chlorambucil (4–6 mg per day) because of generalized disease with severe myopathy. Four patients were maintained on their prior daily doses of prednisone (I. B., 15 mg; Ph. D., 12.5 mg; V. M., 12.5 mg; and E. S., 8 mg) and one on triamcinolone (R. C., 5 mg every other day). Three were continued on 200 mg per day hydroxychloroquine (I. B., E. B., and E. R.), and three of gold injections (G. T., I. B., and E. R.).

Preparation of IgG. The procedures for isolating autologous and homologous (normal) IgG were identical. Sera from five normal volunteers were pooled and used as a source of homologous IgG in seven of the eight studies. In the remaining study, serum from a single volunteer was used. After dialyzing against 0.01 M tris buffer, pH 7.40, the IgG was separated sterilely by chromatography on DEAE-52 cellulose. The IgG was resolved in a single peak and produced single precipitin lines in agar gel diffusion (Ouchterlony) using anti-whole human serum and anti-human IgG, respectively.

Labeling. IgG was labeled with ^{125}I and ^{131}I in 0.1 M NaOH (New England Nuclear, Boston, Mass.) diluted with 0.01 M tris, pH 7.40, by the method of McFarlane as modified by Helmkamp et al. (20, 21). Half of the autologous and homologous IgG was labeled with ^{125}I; the remaining IgG was labeled with ^{131}I. An amount of isotope sufficient to result in a specific activity of 30 μCi/0.5 mg IgG (an average patient dose) was added to 4 mg of IgG and diluted to a total volume of 3.8 cm^3 with 0.01 M tris buffer, pH 7.40. 0.2 cm^3 iodine monochloride was then jetted forcefully into the solution, followed by 0.25 cm^3 of 25% "protective" human albumin. This resulted in not more than two atoms of iodine per molecule of IgG (20, 21). Dialysis of each preparation was performed four times at 4°C using 1,000 cm^3 pyrogen-free normal saline. The solution was sterilized by passage through a 0.45-μm filter. Cultures for bacterial growth and pyrogen tests (22) were negative for all labeled proteins. TCA precipitation showed greater than 98% of the isotope to be protein bound.

Labeled IgG was ultracentrifuged on sucrose density gradients using thyroglobulin (19S), aldolase (8S), and hemoglobin (4.5S) markers. The radioactivity consistently appeared in a single peak (7S), indicating that IgG dimers or other complexes were not present in significant quantities in the samples to be injected.

Study protocol. All patients were injected with autologous and homologous IgG. Six control subjects received autologous and homologous IgG; one received only autologous IgG; and five received only homologous IgG. Two controls (K. H., S. R.) and two patients (E. B., Ph. D.) were studied twice. IgG dosage ranged from 0.3 to 2 mg (4–50 μCi). i.v. injection was followed by aspiration and reinjection of blood to ensure syringe washing. Plasma samples were drawn at 5, 10, 15, 30, and 60 min, at 4, 8, and 24 h, and daily thereafter for 14–21 days. Urine samples were collected at 8, 16, and 24 h and every 24 h thereafter. To block thyroid uptake of radiolabeled iodine, 10 drops of a saturated solution of potassium iodide was given daily to each patient beginning 1 day before the IgG injection. Weekly urinalyses were negative for proteinuria in all subjects.

Data calculation and analysis. Urine and plasma samples were counted simultaneously at the end of the study on an automatic well-type gamma counter. Calculation and graphic presentation of data were performed using isotope counts in the plasma (P) expressed as the $\log_{10}$ percent of injected counts, cumulative urinary excretion of isotope (ΣU) expressed as $\log_{10}$ percent of injected counts, and the extravascular (E) space calculated from: $E = 100 - P - \Sigma U$. Total body isotope retention (P + E) was calculated by summation of P and E. This value also was measured directly by total body counts in one group of subjects. All P curves became linear by day 4 and declined monoexponentially thereafter. The only exception was the P curve for autologous IgG of J. R. which will be discussed below.

FCR equals the percent of labeled protein catabolized per day and was calculated using the integrated rate equations of Nosslin (23), which allow for both intravascular and E catabolism of IgG. This method does not require injections of radiolabeled IgG of equal specific activities and is independent of P volume. It is derived from the basic rate equations:

$$dP/dt = -(k_1 + k_3)P + k_2E, \quad (1)$$
$$dE/dt = k_1P - (k_2 + k_4)E, \quad (2)$$

where P = the plasma or intravascular space, E = the extravascular space, t = time, and the k terms are the rate constants

for exchange between P and E(k_1,k_2), intravascular catabolism (k_3), and E catabolism (k_4) (Fig. 1).

FCRs also were calculated by the method of Campbell et al. (24) for metabolic clearance, which assumes only intravascular catabolism of IgG. The formula for this method is: FCR = U/P, where U = total daily urinary isotope excretion (counts per cm^3 × urine volume) and P = total daily plasma radioactivity (counts per cm^3 × plasma volume). Plasma volume was calculated by dividing the counts in the injected dose by the plasma counts at $time_0$. The latter figure is the Y-intercept of the least squares curve of P disappearance for the first 4 h of study. The metabolic clearance method provides daily FCRs and was used to determine whether there were significant daily changes in FCR during the study period. Except for the autologous study of J. R., to be discussed below, daily FCRs by this method remained relatively constant throughout the study period and were consistent with those calculated by the Nosslin method (23).

IgG synthesis rates (SR) were calculated from the formula: SR = FCR × P_p, where P_p = plasma pool of IgG in milligrams per kilogram, obtained by multiplying the plasma volume (milliliters/kilogram) by the concentration of IgG (milligrams/milliliter) (25).

Since sample sizes were small and prohibit analysis, suggested nonnormal distribution of the FCR data, significance of all results was evaluated by the Wilcoxon ranks sum test (26), except for the mean P- and E-curve slopes which were evaluated by *t* test (27).

Clinical and laboratory determinations. Joint examinations were performed using standard criteria for inflammatory activity. Articular erosions on radiological joint surveys were independently counted by two of the investigators. Extra-articular disease was documented by appropriate clinical criteria. RF was determined using latex reagent (Island Laboratories, Costa Mesa, Calif.). IgG levels were determined by radial immunodiffusion (Mancini) using standard reference sera (Meloy Laboratories, Inc., Springfield, Va.). Immune complexes were quantitated by the Raji cell radioimmunoassay (28). Antinuclear antibodies (ANA) were detected by immunofluorescence (29). ESR were performed by the Westergren method. IgG rheumatoid factor (IgG-RF) was measured by the radioimmunoassay of Carson et al. (30).

RESULTS

Normals vs. RA. A typical graph showing loss of counts from the P, appearance in the E space, and accumulation in the U in a normal control (K. L.) injected with ^{125}I-labeled homologous IgG is shown in Fig. 2. It can be seen that cumulative U excretion of

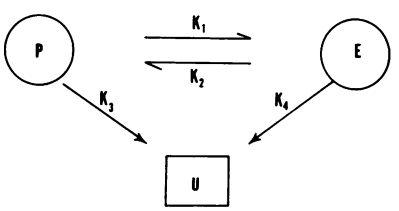

FIGURE 1 Model used for the Nosslin analysis: P, plasma space; E, extravascular space; U, urine = excretory pool (nonexchanging); k_1, k_2, rate constants for exchange between P and E; k_3, rate constant for P-space catabolism; k_4, rate constant for E-space catabolism; (*k* units: percent of labeled protein per day).

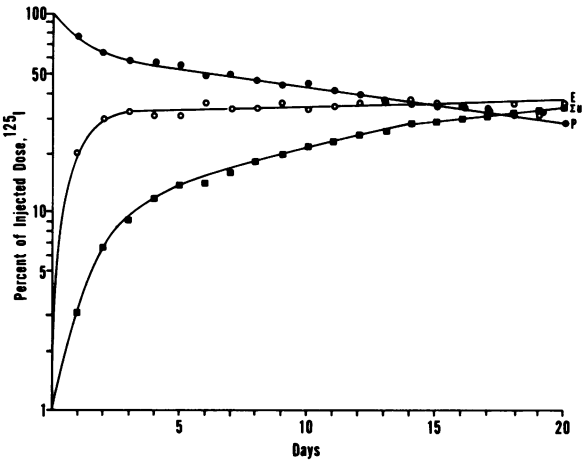

FIGURE 2 Normal metabolism of radioiodine-labeled IgG. P (●), E (○), and Cumulative U (ΣU, ×) radioactivity as percent of injected dose of homo IgG (^{125}I) in control K. L.

isotope reached 35% of the injected dose during the 20-day study period. The 65% remaining in the body at that time was present in the P space (29%) and E space (36%). The curves for the E and P compartments became linear by 4 days. The nearly horizontal E curve specifies sequestration of IgG into an E compartment which has a very slow rate of turnover relative to the P. (See Discussion).

Significant differences were noted between the FCRs for IgG in RA patients and normal controls (Tables I and II). The normal subjects' FCRs for autologous IgG ranged from 2.86 to 5.67% of the total body pool of IgG per day with a mean±SD of 4.44±0.91, and for the homologous IgG from 2.73 to 7.22% with a mean±SD of 4.29±1.26. In the RA patients, FCR for autologous IgG ranged from 4.93 to 14.9% with a mean±SD of 9.67±3.11 ($P < 0.01$), and for the homologous IgG, 3.75–12.5% with a mean±SD of 8.64±2.28 ($P < 0.01$). Mean synthetic rates for IgG also were significantly higher for the RA patients (60 mg/kg per day) than for the controls (14 mg/kg per day) ($P < 0.01$). Thus, the normal to somewhat elevated serum levels of IgG in the RA patients (Table II) are maintained by increased synthesis, which compensates for the high catabolic rate, often overcompensating.

As already noted, from day 4 to the end of the study period, P space curves fell monoexponentially, and daily FCRs calculated by U/P (Campbell method) remained relatively constant. An exception was the behavior of the autologous IgG in patient J. R. In this patient, the P space curve declined biexponentially: more steeply from day 4 to day 8 than from day 8 to day 13. The daily FCRs by U/P varied accordingly, averaging 25% from day 1 to day 7 and 14% from day 8 to day 13. Thus, J. R.'s autologous IgG was metabolized as though it contained two subpopulations, both very

TABLE I
Characteristics of Normal Subjects Studied

Subjects	Age	Sex	Weight	ESR	IgG	FCR (IgG) Auto	FCR (IgG) Homo	FCR difference Auto − Homo
	yr		*kg*	*mm/h*	*mg/ml*	*%/day*		
E. K.	39	M	71	9	9.0	5.67	—	—
C. C.	23	F	56	5	10.0	—	7.22	—
R. D.	51	F	59	10	6.6	—	4.96	—
K. L.	21	M	80	7	7.6	—	3.62	—
J. H.	32	F	47	7	7.6	—	3.90	—
H. S.	48	M	100	5	11.3	—	2.73	—
K. H.*	34	F	65	5	6.0	4.28	4.98	−0.70
K. H.*	34	F	65	8	6.0	4.45	3.62	+0.83
A. M.	32	F	53	9	9.8	2.86	4.13	−1.27
S. R.*	35	F	55	6	9.1	—	3.19	—
S. R.*	35	F	55	6	8.6	4.64	—	—
S. M.	33	M	73	1	7.5	4.71	4.55	+0.16
Mean±SD	35±8.6		65±14	7±2	8.3±1.7	4.44±0.91	4.29±1.26	+0.15

* Studied twice.

TABLE II
Characteristics of RA Subjects Studied

Subjects	Age	Sex	Weight	ESR	IgG	Latex titer	ANA titer	Immune complexes*	Erosions on X ray	Duration of disease symptoms	Active joints‡	Extra-articular disease	FCR (IgG) Auto	FCR (IgG) Homo	FCR difference Auto − Homo
	yr		*kg*	*mm/h*	*mg/ml*					*yr*	*number*	*No. of manifestations§*	*%/day*		
Pa. D.	51	F	52	25	8.5	2,560	16	133	+	18	4	1	4.93	4.92	+0.01
E. S.	79	F	36	96	18.0	5,120	4	28	+	8	18	0	5.32	5.95	−0.63
G. T.	66	F	69	40	16.6	320	0	107	+	15	5	0	6.92	9.63	−2.71
G. B.	70	F	50	38	9.7	2,560	64	73	−	35	5	1	7.07	3.75	+3.32
Ph. D.‖	48	F	67	88	9.4	2,560	64	110	+	13	0	1	7.17	9.29	−2.12
I. B.	57	F	65	20	6.8	5,120	16	43	−	2	4	0	7.27	7.12	+0.15
E. R.	58	F	68	30	13.5	640	16	17	+	6	10	1	7.58	8.05	−0.47
J. U.	50	F	54	45	15.9	640	0	268	+	12	6	0	7.68	7.79	−0.11
V. M.	52	F	62	40	8.9	2,560	0	4	−	20	18	0	8.08	8.58	−0.50
P. F.	61	F	57	41	13.0	640	64	211	+	2	3	1	8.68	8.82	−0.14
Ph. D.‖	48	F	67	63	8.2	1,280	256	26	+	13	1	1	10.7	7.25	+3.45
C. A.	75	F	50	50	16.0	5,120	256	175	+	4	50	2	11.0	10.2	+0.80
F. R.	38	F	58	20	20.5	1,280	64	0	−	3	13	1	11.1	6.97	+4.13
R. C.	63	M	87	38	9.0	10,240	256	26	−	24	0	1	11.9	12.2	−0.30
V. C.	54	F	66	31	14.0	1,280	64	94	+	20	26	2	12.1	9.93	+2.17
T. J.	47	M	64	93	15.5	2,560	0	38	+	9	0	2	13.4	10.1	+3.30
E. B.‖	72	F	62	65	22.6	20,480	256	1,000	−	2	0	3	13.7	8.38	+5.32
E. B.‖	72	F	62	108	22.5	5,120	256	1,000	−	2	0	3	14.3	11.6	+2.70
V. R.	72	F	62	40	13.5	5,120	0	95	+	3	19	0	14.9	9.85	+5.05
J. R.	71	F	49	95	9.7	5,120	4	118	+	25	37	3	¶	12.5	—
Mean ±SD	60 ±11		60 ±10	53 ±28	13.6 ±4.8								9.67 ±3.11	8.64 ±2.28	+1.03

* Micrograms of aggregated human globulin equivalents (28).
‡ Tenderness ≥ two on a scale of four, plus swelling.
§ Include: sicca, Felty's, vasculitis, nodules, pleural effusion, myopathy, neuropathy.
‖ Studied twice.
¶ 14–25% (see Results).

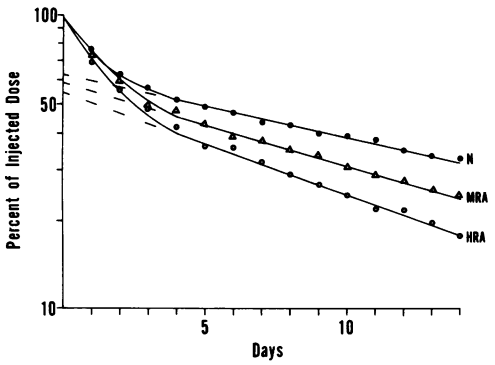

FIGURE 3 Mean curves of plasma decay for homo IgG in normals (N, ●), MRA (△), and HRA (○). Derivation is by least squares analysis of mean data for days 4–14. *P* values for slopes: N vs. MRA, $P < 0.01$; N vs. HRA, $P < 0.001$; and MRA vs. HRA, $P < 0.01$.

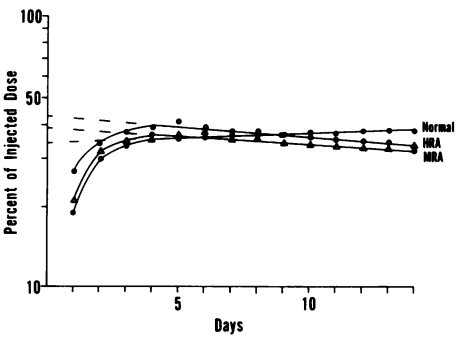

FIGURE 4 Mean curves for E homo IgG: symbols and derivation are as for Fig. 3. *P* values for slopes: N vs. MRA, $P < 0.001$; N vs. HRA, $P < 0.001$; and MRA vs. HRA, NS.

rapidly catabolized but one much more so than the other. This patient had severe extra-articular disease (nail fold thrombi, myopathy, erythema multiforme, and sicca syndrome). She also had the most markedly elevated Factor B and C3 turnover rates seen in a review of RA patients to be reported separately.[2] These unusual metabolic observations obviously may be highly pertinent to the clinical aspects noted (see Correlations, below). However, since her turnover data for autologous IgG were unique in this seies, we excluded them from all composite calculations.

The RA patients arbitrarily were divided into two groups on the basis of FCR for autologous IgG. Nine patients had autologous FCR greater than 10% (Table II) and were designated highly hypercatabolic RA (HRA). The remainder of the patients were designated mildly hypercatabolic (MRA). It was striking to note that within the HRA group, the mean FCR for autologous IgG (12.6±1.57) was significantly higher than that for homologous IgG (9.90±1.92) ($P < 0.01$).

[2] Krick, E. H., D. DeHeer, J. H. Vaughan, C. M. Arroyave, and R. A. Kaplan. Manuscript in preparation.

The linear portions (day 4 to day 14) of the mean P curves for homologous IgG in the two RA groups and the normals were calculated by the least squares method. Disappearance of radiolabeled homologous IgG from the P space (Fig. 3) was significantly increased in the HRA compared to MRA patients ($P < 0.01$), which, in turn was significantly faster than the normal ($P < 0.01$). This difference parallels the differences in FCR by which the groups were selected. The enhanced clearance of the homologous IgG from the P space in RA could be due to increased intravascular catabolism, to increased movement into the E space, or to both.

Fig. 4 depicts the mean E-space curves. IgG appeared in the E space earlier and attained higher maxima (days 1–5) in both the HRA and MRA groups as compared to the normals. Additionally, the IgG was eliminated more rapidly from the E space in the RA patients, particularly the HRA group, as indicated by the slopes of the lines from day 5 to day 14 ($P < 0.001$). These graphic expressions of E-space dynamics were corroborated by the rate constants calculated by Nosslin's equations (Fig. 1, Table III). The ratio of the exchange rates between P space and E space (k_1/k_2) was significantly increased ($P < 0.01$, homologous [homo]; $P < 0.05$, autologous [auto]) in the HRA group versus

TABLE III

Rate Constants for Movement of IgG between P and E Spaces and for its Catabolism as Calculated by Nosslin's Method

	k_1/k_2		k_3*		k_4*	
Group	Auto	Homo	Auto	Homo	Auto	Homo
Normals	0.61±0.44	0.76±0.49	5.57±3.02	4.62±3.34	−2.71±6.80	−0.15±3.62
MRA	0.98±0.38	0.96±0.36	5.15±2.03	5.13±1.85	2.03±3.05	3.06‡±3.20
HRA	1.22‡±0.27	1.31‡±0.31	6.19±1.42	5.67‡±1.48	6.48‡§‖±1.73	3.93‡±2.49

* Percent of labeled protein per day (mean±SD).
‡ Statistically significant vs. normals.
§ Statistically significant vs. MRA.
‖ Statistically significant vs. homo value for same group. See text for *P* values.

the controls, indicating an increased net movement of IgG from P to E. This was more marked in the HRA than the MRA group. Catabolism of IgG in the E space (k_4) was evident in the RA patients, especially the HRA patients, and was seen with both homo and auto preparations (MRA, homo, $P < 0.05$; HRA, homo, $P < 0.01$; HRA, auto, $P < 0.001$). No catabolism of IgG occurred in the E space in the normals. By contrast, P space catabolism increased little or not at all in the RA patients. These findings suggest that: (*a*) there is enhanced P to E movement of IgG in RA; and (*b*) most of the hypercatabolism of IgG in RA occurs in the E space.

Auto IgG vs. homo IgG. As noted, the HRA group catabolized auto IgG faster than homo IgG ($P < 0.01$). This group's mean E-space curve for auto IgG declined more rapidly ($P < 0.001$) than did that for homo IgG (Fig. 5). This was not true for the HRA group's mean P-space curves (Fig. 6). Similarly, the k_4 for auto IgG was higher ($P < 0.01$) than that for homo IgG, whereas this was not true for the k_3 values (Table III). Thus, the increased FCR of auto over homo IgG seems to be attributable entirely to events in the E space.

IgG-RF was measured in the auto IgG preparations of six HRA's and three controls. Each HRA and none of the control preparations was positive (Table IV). In both groups, the daily FCR (U/P) was consistent through study days 1–10 (Table IV). These results are discussed below (see Discussion).

E-space calculation. The calculation of E space depends upon completeness of U collection and lack of any other loss of IgG or its breakdown products. No patient had gastrointestinal or renal disease. One HRA patient, two MRA patients, and one control underwent weekly extracorporeal total body counting[3] during otherwise standard turnover studies. The total body counts derived in this way corroborated the calculated quantities for residual total body isotope using P + E. Thus, the validity of the method of calculating P + E, and therefore of E, was confirmed for the current study.

Correlations with clinical and laboratory indices. Eight of the nine HRA patients exhibited a total of 15 manifestations of extra-articular rheumatoid disease, whereas only 5 of the 10 MRA patients exhibited such features, 1 in each case ($P < 0.001$). The extra-articular manifestations included nodules, sicca syndrome (with positive Schirmer test), Felty's syndrome, vasculitis (active or clearly documented by history), neuropathy, myopathy, and pleural effusion (Table II). Although E. B. was studied twice, her extra-articular

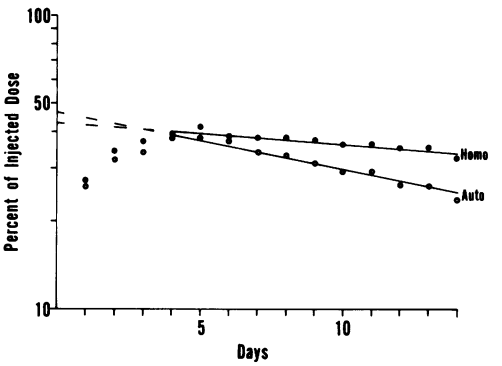

FIGURE 5 Mean curves for E Auto (○) and Homo (●) IgG in the HRAs. Derivation is by least squares analysis of mean data for days 4–14. *P* value for slopes <0.001.

manifestations are counted only once. Ph. D., who had a highly elevated FCR once and a moderately elevated FCR once, was counted separately in each group. The mean and median latex titers, ANA titers, and assays for immune complexes were higher in the HRA than the MRA group, but the overlap was considerable (Table II). We could not distinguish the HRA from the MRA group on the basis of age, weight, sex distribution, duration of disease (by symptoms), serum IgG concentration, number of active joints, number of erosions on X ray, or ESR.

DISCUSSION

Metabolism of homo IgG. Our results agree with the previous reports (5, 6, 10, 11, 16) of an increased rate of IgG catabolism in RA patients as compared to normal individuals. The factors responsible for hypercatabolism of IgG in RA are not known. Wochner (16) noted that IgG is rapidly catabolized in a variety of the inflammatory diseases of the connective tissues. In addition to RA, he studied systemic lupus erythematosus, polymyositis, and vasculitis. He could not relate degree of hypercatabolism

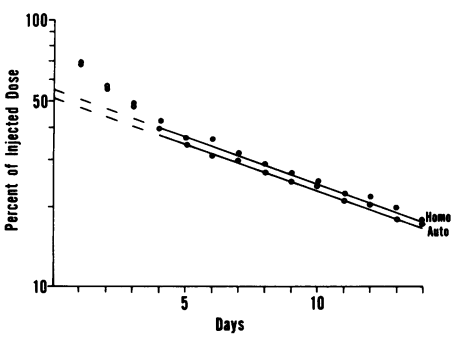

FIGURE 6 Mean curves of plasma decay in the HRAs. Symbols and derivation are as for Fig. 5. *P* value for slopes is not significant.

[3] We are indebted to Dr. W. H. Blahd of the Wadsworth Veterans Administration Hospital, Los Angeles, Calif., for use of the total body counter in that insitituion.

TABLE IV

*Daily FCR for Auto IgG of Six HRA Patients and Three Normal Controls**

Subject	IgG-RF	Daily U/P (percent of labeled protein per day)									
		1	2	3	4	5	6	7	8	9	10
HRAs											
V. R.	+	8.4	10.1	12.8	20.9	15.8	12.2	12.2	28.7	17.3	12.3
R. C.	+	7.7	10.8	14.3	12.0	12.5	17.8	11.8	12.2	10.2	12.8
Ph. D.	+	6.1	10.7	12.5	9.1	10.5	11.9	11.3	10.7	13.2	9.1
T. J.	+	5.6	11.4	13.7	13.9	13.8	14.4	16.4	15.9	16.3	16.0
V. C.	+	7.0	12.0	11.8	16.2	13.4	14.5	12.2	13.6	10.6	12.4
E. B.	+	12.0	10.1	13.7	13.1	10.7	21.5	15.6	15.9	17.7	15.2
Controls											
S. R.	−	7.3	5.8	6.4	4.3	4.2	6.1	4.2	4.5	4.2	4.1
S. M.	−	7.6	5.2	4.0	4.2	4.9	4.5	4.3	4.4	4.1	4.9
K. H.	−	4.4	4.2	3.8	3.9	4.6	6.4	4.4	4.4	4.5	4.9

* Calculated by metabolic clearance method (U/P). Presence (+) or absence (−) of IgG-RF in the auto IgG is noted.

to serum RF. There was no bulk loss of protein via the kidneys or gastrointestinal tract. General hypercatabolism of body proteins was not present, as evidenced by normal albumin metabolism in his patients. The IgG hypercatabolism was specific for connective tissue diseases and was not present in 15 "disease controls" with various chronic, inflammatory, often debilitating illnesses not due to connective tissue diseases.

Age has been shown to contribute to differences in serum half-life of IgG in children. After adulthood, however, this variation apparently ceases (31). We found no correlation between age and FCR for either our normals or our RA patients.

Prednisone, in doses of 30 mg per day, was found by Griggs et al. (32) to increase FCR from a mean of 7.4 to 9.5. Others have not observed this effect (16, 33). Five of our patients were on corticosteroids, four were on prednisone (maximum of 15 mg per day), and one was on triamcinolone (5 mg every other day). The FCR elevations noted in this study cannot, therefore, be attributed to a corticosteroid effect.

Waldmann and Jones (34) have noted a serum concentration-dependent catabolic effect on IgG metabolism. However, several studies which demonstrated hypercatabolism of IgG in patients with RA and with other connective tissue diseases found no correlation with serum IgG levels (6, 11, 16). Likewise, linear regression analysis of our data indicated no significant correlation between serum IgG level and FCR, plotted linearly or semilogarithmically.

Incorporation of the injected IgG into immune complexes could hasten its elimination by precipitation in tissues and by reticuloendothelial clearing. Sera from our subjects were assayed by the Raji cell technique for complement-fixing, circulating immune complexes (28). Significant amounts (>25 μg aggregated human globulin equivalents per ml) were detected in all but four patients (R. C., V. M., F. R., and E. S.) (Table II). There was no significant difference between the HRA and MRA groups. Thus, whereas immune complexes as measured by the Raji cell assay do exist in the circulation in RA, their presence seems insufficient to explain the increased catabolism of IgG. Alternate techniques for detection and quantitation of circulating immune complexes, including methods not dependent on complement fixation, should be tried to determine whether they will yield positive correlations with IgG FCR. In addition, one must recognize that E antibody production could account for significant quantities of immune complexes which never reach the circulation. RF complexes must be similarly considered. Vaughan et al. (35), using an assay of hemolytic plaque-forming cells, detected RF-producing lymphocytes in the joint fluids of some RA patients, indicating that these cells can migrate through the interstitium. Furthermore, the RF so detected reacts much more avidly with IgG than does serum RF. Thus RF, as it is immediately released into the interstitium, may interact more strongly with IgG and play a unique role in IgG metabolism.

Metabolism of auto IgG. The FCR for auto IgG was significantly greater than for homo IgG in the HRA group ($P < 0.01$). In previous studies in RA, no difference between auto and homo IgG metabolism was reported (13, 16). Simultaneous measurements were not made, however, in the study by Vaughan et al. (13). In the study by Wochner (16) no data were given, only a general statement about the issue.

One possible explanation for the increased FCR of auto IgG is that this material may have contained unusual quantities of IgG_3, a subtype known to be

catabolized more rapidly than other subtypes (36–38). We used an anti-IgG_3 serum[4] to assay the IgG_3 content of the IgG preparations from six HRAs, three controls, and the normal pool. In no case did we find quantities of IgG_3 that could account for the observed FCRs.

Although complexes were not detected by ultracentrifugal analysis of the labeled IgG preparations, it is conceivable that the auto preparations from the RA patients contained low avidity IgG-RF and that this might have been metabolized more rapidly. The auto preparations of six HRAs did indeed contain IgG-RF by the radioimmunoassay of Carson et al. (30) (Table IV). However, by the metabolic clearance method of calculating daily FCRs (U/P, see Methods), the hypercatabolism of auto IgG in the six HRA patients persisted at a consistent level through at least 10 study days (i.e., for as long as daily urinary counts sufficed to calculate U/P). By that time, a mean of 47% of auto IgG had been eliminated. The increased catabolism, therefore, must have involved at least the majority of the molecules in the preparation, and probably all of them. It is highly unlikely that a majority of the injected IgG molecules were IgG-RF.

There is some evidence that RFs react preferentially with auto IgG in patients with rheumatoid disease (39, 40). Five genetically determined sites on IgG have been defined at which RF may react (41). They occur on the Cγ2 and Cγ3 domains of the Fc fragment and are variously distributed among the IgG subtypes. The increased catabolism of RA IgG over normal IgG may reflect the presence of more RF-reactive sites on the former, resulting in RA IgG-RF complexes and enhanced catabolism. This possibility will be explored separately.

Some evidence has suggested biochemical differences between RA and normal IgG (15, 17, 18). Johnson et al. (17, 18) reported a slight change in the circular dichroism spectrum of the RA IgG molecule. They postulated an increased avidity of RA IgG for RF, thus accelerating IgG turnover. We did not find a change by circular dichroism[5] in the IgG of J. R. or E. B., as compared to one normal control and the pooled normal IgG. Watkins et al. (15) observed enhanced catabolism of RA IgG in mice and suggested an "immune mechanism" type of clearance. We have initiated similar studies in rabbits, which have been inconclusive thus far. Mullinax (42) has noted a selective galactose deficiency in IgG from patients with RA and systemic lupus erythematosus. We plan to analyze the saccharide contents of the IgG preparations in our study.

The preponderance of extra-articular disease which we have observed in the HRA group is intriguing and previously unreported. This clinical-metabolic concurrence may define a subset of RA patients with pathogenetic mechanisms distinct from those operative in patients with fewer extra-articular manifestations and lower FCRs. However, the observed quantitative differences do not seem to justify such a qualitative distinction. Rather, it seems likely to us that all RA patients preferentially hypercatabolize auto IgG by mechanisms central to their underlying "autoimmune" disease process, but current methods discern this phenomenon only in the more highly hypercatabolic patients.

It will be of interest to study other connective tissue diseases for comparative metabolism of auto and homo IgG.

E catabolism. Nosslin's mathematical method of kinetic analysis distinguishes between intravascular (P-space) and extravascular (E-space) catabolism. To our knowledge, such information has been reported in only one other study of immunoglobulin metabolism, which compared the turnover of M-components and normal IgG in plasma cell dyscrasias (43). It should be emphasized that E space does not specify an anatomical locus. Rather, it indicates all physiological compartments, other than intravascular, in which the labeled protein is distributed.

Our results emphasize that much or most of the increased catabolism of IgG in RA occurs in the E space. In normal individuals, IgG catabolism is exclusively intravascular. This is verified in our normal controls by the low or negative value for k_4 (Table III). By contrast, E space in RA is in a heightened state of metabolism, represented by positive k_4 values and negative E-curve slopes.

The nature of the E-space compartments in which IgG is catabolized in RA (Figs. 4 and 5) is not well characterized. The vascular endothelium can conceivably adsorb or incorporate IgG which would thereby become inaccessible to plasma (P-space) sampling. The E space may include a number of surfaces which bind IgG via Fc receptors like those that have been demonstrated on lymphocytes, neutrophils, and monocytes (44, 45). Other cells are undoubtedly involved because less than 1% of the IgG bound in E is accounted for by combining the estimated total numbers of circulating leukocytes and fixed macrophages with their IgG-binding capacities (45–50). Waldmann and Strober (51) have found evidence of Fc receptors in both the gut and eviscerated carcasses of mice.

Brambell (52) has postulated "protective sites" in his scheme for the normal catabolism of IgG. What relation the slowly metabolizing compartment in the E space

[4] Kindly provided by Dr. Hans Spiegelberg, Department of Immunopathology, Scripps Clinic and Research Foundation, La Jolla, Calif.

[5] We thank Dr. William Morgan from the Department of Biochemistry, Scripps Clinic and Research Foundation, La Jolla, Calif. for these assessments.

would have to such protective sites is uncertain. Hypercatabolism of IgG in the E space of Ra patients might represent an increased turnover of receptor sites or their conversion into catabolic sites. The existence of such receptors in the E space is purely inferential at the present time, but relevant information on this question may come from simultaneous studies of IgG turnover and the Fc receptor dynamics of normal and RA peripheral leukocytes.

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