

Production of Antibodies Specific for Fc, Fab', and Streptokinase-Streptodornase In Vitro by Peripheral Blood Cells from Patients with Rheumatoid Arthritis and Normal Donors

IDENTIFICATION OF IMMUNE COMPLEXES IN CULTURE SUPERNATANTS CONTAINING HIDDEN ANTIBODIES REACTIVE WITH Fab' FRAGMENTS OF IMMUNOGLOBULIN G

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ABSTRACT To study antibody (Ab) biosynthesis in rheumatoid arthritis (RA), the immunoglobulin (Ig)M anti-Fc, anti-Fab', and antistreptokinase-streptodornase (SKSD) produced by peripheral blood lymphocytes (PBL) were measured at intervals from 1 to 19 d in culture. PBL from 17 seropositive patients with active RA and 30 age-matched controls were evaluated. Within the first 24 h, PBL from six of eight patients released >30 ng IgM anti-Fc, even in the absence of pokeweed mitogen (PWM). This early release of Ab was blocked by cycloheximide. With or without PWM, PBL from normal donors did not release IgM anti-Fc until after 3–5 d in vitro. By day 9, unstimulated PBL from seven patients made >100 ng IgM anti-Fc. Unstimulated PBL from normals never made >95 ng of this Ab.

When PWM was added, PBL from normal donors released as much IgM anti-Fc as was found in RA donor cultures. Paradoxically, addition of PWM to PBL of RA patients suppressed release of IgM anti-Fc in 4 of 17 cases to levels significantly below those found in unstimulated cultures of the same cells.

Without PWM, PBL from RA donors frequently

failed to make IgM anti-SKSD ($P < 0.05$ compared with normal donors' cells). With PWM, the quantities of IgM anti-SKSD released were comparable.

Fluctuations in IgM anti-Fab' levels during the lifetime of these cultures were sufficient to suggest that these Ab may be taken up in immune complexes. This hypothesis was verified by acidifying (pH 3.1) culture supernatants to which ^{125}I -Fab' had been added previously. The samples were then neutralized (pH 7.6) and 12% polyethylene glycol was added to separate free from antibody-bound ^{125}I -Fab'. This procedure increased the quantity of ^{125}I -Fab' precipitated by >10-fold in some cases.

These studies suggest that there are a variety of abnormalities in Ab biosynthesis in RA. These include spontaneous synthesis of comparatively large quantities of IgM anti-Fc, relatively suppressed release of IgM anti-SKSD, and a paradoxical reduction, in some cases, in the biosynthesis of IgM anti-Fc after addition of PWM.

INTRODUCTION

Antiimmunoglobulin (anti-Ig) production is frequently observed in rheumatoid arthritis (RA)¹ and

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¹Abbreviations used in this paper: Ab, antibody; Ag, antigen; FCS, fetal calf serum; PBL, peripheral blood leuko-

other chronic diseases (1–3). Cultured peripheral blood lymphocytes (PBL) from healthy donors can also produce anti-Ig when stimulated with pokeweed mitogen (PWM) or Epstein-Barr virus, (4–7). This suggests that synthesis and release of these Ab can reflect a normal, appropriate response to certain stimuli. Conceivably, the very high levels of anti-Ig found in RA may represent a normal physiological reaction to unusually strong or persistent antigenic stimulation. Alternatively, there may be abnormalities in immune regulation that facilitate formation of anti-Ig in RA. These abnormalities may be selective, with the result that synthesis of other Ab remains relatively normal. Or there may be multiple immunoregulatory defects in RA. The latter might help to explain some of the other abnormal serologic features of this disease, most notably, polyclonal hypergammaglobulinemia, circulating immune complexes, and the formation of anti-nuclear and other autoantibodies (8). To evaluate Ab biosynthesis in RA, the quantities of IgM anti-Fc, IgM anti-Fab', and antistreptokinase-streptodornase (SKSD) released by PBL from patients and age-matched controls were monitored at frequent intervals for up to 19 d in culture.

METHODS

Donor population. 17 patients between 39 and 70 yr (mean age = 58 yr) with active classical or definite seropositive rheumatoid arthritis, as defined by the Ropes criteria (9), were studied; 15 were male. All were taking nonsteroidal antiinflammatory drugs. In addition, three were receiving corticosteroids, six, gold injections, and five, D-penicillamine. 30 healthy donors, aged 19–86 (mean age = 54 yr), of which 15 were male, served as controls.

Lymphocyte culture. PBL were isolated from heparinized peripheral blood by means of Ficoll-Hypaque. Cells were washed three times with Hanks' balanced salt solution (HBSS), four times with fetal calf serum (FCS), and once with RPMI 1640 (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.). Differential counts revealed 57–81% lymphocytes (mean \pm SD for RA patients, 68 ± 10 ; for normals, 74 ± 10), 13–35% monocytes (RA patients, 31 ± 14 ; normals 21 ± 9), and 1–13% PMN (mean 7%). Cells were adjusted to 1×10^6 lymphocytes per milliliter in RPMI 1640 supplemented with 100 mM L-glutamine, 10% FCS, 100 U/ml penicillin, and 100 μ g/ml streptomycin. In addition, each individual's cells were cultured with and without PWM (Gibco Laboratories). Dose-response studies using concentrations between 0.25 and 2.0% indicated that a dose of 1% PWM was optimum to promote specific Ab synthesis by PBL from normal and most RA donors. Cycloheximide (100 μ g/ml) was added to some cultures to block protein synthesis. Cultures were distributed in 1-cm³ aliquots to 12 \times 75-mm polystyrene tubes and maintained in 5% CO₂ at 37°C. At the end of 1–19 d, cells were spun and the supernatants were decanted. Replicate supernatants were pooled and stored at

–20°C until used. Freeze/thaw lysates provided a day zero estimate of the quantity of Ab carried over in and on the cells.

Radioimmunoassay (RIA). Specific Ab was measured by a solid phase RIA modified from Carson et al. (10). Antigens included SKSD and the Fc and Fab' fragments of human IgG. Culture supernatants were diluted 1:2 with 1% BSA in Tris saline, pH 7.4, before testing in RIA. ¹²⁵I-rabbit anti-human IgM was added to detect bound Ab. The range of triplicates fell within 10% of the mean. A background value, representing the uptake of the labeled anti-IgM by Ag and the bovine serum albumin (BSA)-coated tube alone, was subtracted from each estimation of specific Ab. A standard curve was prepared by coating increasing quantities of IgM onto polystyrene tubes. The IgM incorporated a trace radiolabel to allow determination of the amount of IgM adherent to the tube. Sites not occupied by IgM were blocked by adding Tris saline containing 1% BSA. ¹²⁵I-anti-IgM was then added, and after overnight incubation at 4°C, the tubes were washed and counted. The counts per minute of ¹²⁵I anti-IgM bound was plotted as a function of the amount of IgM known to be adherent to the tube. This gave identical results as a standard curve prepared using serial dilutions of specifically purified human IgM anti-Fc assayed in Fc-coated tubes. Assays were reported as nanograms of specific Ab bound to the antigen-coated tube. For some studies, the RIA was modified to test 100- μ l samples in microtiter plates. Results of these assays were recorded as nanograms of Ab bound per 100 μ l of sample. IgM levels were measured in a similar solid-phase RIA using polystyrene tubes coated with specifically purified rabbit anti-IgM.

Measurement of "hidden" or "blocked" antibodies reactive with Fab'. 100 μ l of culture supernatant was mixed in triplicate with 100 μ l of ¹²⁵I-Fab'. The ¹²⁵I-Fab' had been spun at 40,000 g for 30 min to remove aggregates and then adjusted to 1.06×10^6 cpm/100 μ l in RPMI 1640 containing 200 μ g/ml trasylol, 2 mM phenyl methyl sulfonyl fluoride, 0.02% sodium azide, and 10% FCS. The pH was decreased to 3.1 with citric acid. The samples were incubated for 30 min at 37°C, then for 30 min more at room temperature (23°C). 3 M Tris base was then added to restore pH to 7.6. Control aliquots of supernatant plus ¹²⁵I-Fab' remained at neutral pH throughout and received borate-buffered saline to bring them to the same volume. The samples were incubated overnight at 4°C. Polyethylene glycol (PEG 6000, Fisher Scientific Co., Pittsburgh, Pa.) was added to a final concentration of 12%. The samples were incubated at 0°C for 60 min and spun at 1,500 g for 30 min at 0°C. The supernatants were decanted, and the precipitated radioactivity was counted. Data were corrected for the amount of Fab' precipitated by the PEG in the absence of specific Ab. To evaluate the ability of the modified Farr assay to detect soluble Fab'/anti-Fab' complexes, rabbit anti-Fab' of known Ab content was added to culture media containing ¹²⁵I-Fab' at molar Ag/Ab ratios ranging from 1:17 to 150:1. 12% PEG was adequate to bring down >85% of the soluble complexes formed in less than threefold molar Ag excess and >19% of the complexes formed in 3–30-fold M Ag excess. To demonstrate that the acidification step dissociated immune complexes, comparison assays were carried out in which the culture supernatant and ¹²⁵I-Fab' were acidified and neutralized independently and then combined just before the PEG precipitation step (see Table IV).

Reagents. Human IgG was purified from Cohn fraction II (Pentex, Miles Laboratories, Inc., Elkhart, Ind.) by means of DEAE Sephacel (Pharmacia Fine Chemicals, Div. Pharmacia Inc., Piscataway, N. J.). Fc was prepared by digestion

cytes; PEG, polyethylene glycol; PWM, pokeweed mitogen; RA, rheumatoid arthritis; RIA, radioimmunoassay; SKSD, streptokinase-streptodornase.

with papain (11). Fc and undigested IgG were segregated from Fab fragments by absorption and elution from Protein A Sepharose. The 50,000-mol wt Fc fragment was separated from intact IgG by S-200 (Pharmacia Fine Chemicals) filtration, and passed over a column of rabbit anti-Fab' immobilized on CNBr-Sepharose (Pharmacia Fine Chemicals) to remove the last traces of Fab. This protein, at 1 mg/ml, formed a precipitin band with anti-Fc but failed to react with assorted anti-Fab' antisera by double diffusion in agar. For preparation of Fab' the IgG was digested with pepsin (11). Undigested IgG was removed by passing the mixture over Protein A Sepharose. The effluent was reduced and alkylated (11). The 50,000-mol wt peak was collected by S-200 filtration. This preparation reacted with anti-Fab' antisera by double diffusion in agar, but failed to react with assorted anti-Fc antisera.

SKSD (Varidase, for intramuscular use) was purchased from Lederle Laboratories, Div. American Cyanamid, Wayne, N. J. IgM was isolated from normal human serum by the method of Masseyeff et al. (12), using $MnCl_2$ and heparin to remove β -lipoproteins (13). By immunoelectrophoresis the isolated protein gave a single precipitin line characteristic of IgM. This IgM anti-IgM immunoprecipitate, after repeated washings, was used to prepare antiserum in rabbits. Cross-reacting Ab to IgG were removed on IgG Sepharose. When radiolabeled and used in the RIA, this antiserum detected 1 ng of immobilized IgM but would not bind to 5,000 ng immobilized IgG. Anti-IgM used in the RIA was specifically purified by adsorption and elution from IgM Sepharose, digested to the $F(ab')_2$ fragment by means of pepsin, and radiolabeled by the chloramine T method (14).

Specific IgM anti-Fc was isolated from the serum of a single seropositive RA patient by affinity chromatography on human IgG-Sepharose. Anti-IgG was eluted with 1 M acetic acid. Antibodies specific for Fab' were removed by adsorption with Fab'-Sepharose, leaving only anti-Fc.

Specificity of the RIA. Addition of soluble IgG in concentrations ranging from 1,000 to 10,000 ng/ml (the range typically observed in culture supernatants) did not interfere with the quantitation of antiimmunoglobulins in the supernatants. The three Ab were measured specifically and independently. Data in Table I indicate that adsorption of one Ab did not decrease the measured quantities of others. Addition of extra anti-Fc increased only the quantity of IgM anti-Fc detected (Table II). These experiments were done to determine whether the high levels of IgM anti-Fc present in some culture supernatants could falsely elevate the measurements of IgM Ab to other Ag. The culture supernatants, as mentioned, also contained IgG. Had these bound specifically or nonspecifically to the Ag-coated tubes in sufficient quantity, they might have created an adsorptive surface that would bind IgM anti-Fc Ab. In practice, this was evidently not a problem.

To evaluate whether the Ab measured in the RIA was 19S IgM, or included large soluble complexes formed in Ab excess, culture supernatants from two donors were fractionated by sucrose density gradient ultracentrifugation. Ab was not found in fractions sedimenting more rapidly than 19S.

RESULTS

Kinetics of antibody formation in vitro. The rate of release of IgM anti-Fc, anti-SKSD and anti-Fab' by cultured PBL from a representative RA, and a normal donor are shown in Fig. 1. In the normal donor's culture, IgM anti-Fc was first detected after day 3. Ab

levels rose to a maximum by day 9. In the absence of PWM, the Ab rose more slowly than in corresponding PWM-driven cultures. Release of IgM anti-SKSD and anti-Fab' followed a similar time-course.

The kinetics for IgM anti-Fc release by PBL from the RA patient were strikingly different. 24 h after establishing the culture, >30 ng of IgM anti-Fc were present. The quantity of IgM anti-Fc increased without delay. This accelerated release of IgM anti-Fc was observed in 15 of the 17 RA patients.

The kinetics of anti-SKSD release were also abnormal. Ab levels increased without delay in PWM-stimulated cultures. This was not a consistent feature, however, of RA donor cultures; a third of these patients exhibited accelerated kinetics, others showed either normal or even excessively extended lag periods before IgM anti-SKSD was detectable in culture supernatants. Without PWM, this donor's cells made very little IgM anti-SKSD, even though they produced abnormally great quantities of IgM anti-Fc.

Anti-Fab' levels fluctuated in a manner not observed with IgM anti-Fc or anti-SKSD. After a brisk increase, anti-Fab' fell progressively in cultures from the RA patient harvested after day 3. The anti-Fab' activity of his unstimulated cultures exceeded that in supernatants of PWM-stimulated PBL for the first 9 d in vitro (Fig. 1).

Cultures of PBL from additional normal donors (Fig. 2) displayed kinetics similar to those shown in Fig. 1. However there was extensive variability in the response of PBL from eight RA patients studied concurrently. Six of the eight had high levels of IgM anti-Fc on day 1 (Fig. 2A), and only two patients showed any delay before the rise in IgM anti-Fc activity. By day 11, the magnitude of the anti-Fc response ranged from 35 to 200 ng reflecting increases of 10–140 ng over the quantities present on day 1.

In the absence of PWM there was a minimal anti-SKSD response in the RA patients. Four failed to make detectable quantities of anti-SKSD by day 11. PWM induced IgM anti-SKSD release from PBL of most RA patients within 1–5 days, but the Ab levels increased less quickly and less smoothly than in cultures from normal donors (Fig. 2B).

Anti-Fab' levels in these cultures rarely showed the smooth asymptotic increments characteristic of anti-Fc and anti-SKSD release in vitro (Fig. 2C). The day to day fluctuations in anti-Fab' greatly exceeded the confidence limits of the assay (Fig. 1) and thus indicated true fluctuations in the amounts of anti-Fab' detected. With the addition of PWM, PBL from normal donors produced a steady increase in anti-Fab' but the kinetics of release in RA patients' cultures still fluctuated widely.

TABLE I
Effect of Selective Adsorption of Culture Supernatants with Insoluble Fc, Fab', or SKSD
on Measurement of Other Antibody Activities

Donor	Culture supernatant previously adsorbed with:†	Antibody activity*			
		IgM anti-Fc		IgM anti-Fab'	
A	Fc	316±8	(66%)§		
	SKSD	484±3	(101%)	ND	
	BSA	478±1	(100%)		
B	Fc	648±9	(15%)	1,575±65	(87%)
	Fab'	3,984±78	(91%)	1,001±70	(55%)
	BSA	4,357±68	(100%)	1,814±199	(100%)
C	Fc	311±12	(6%)	181±12	(106%)
	Fab'	5,470±289	(97%)	76±13	(44%)
	BSA	5,629±148	(100%)	171±17	(100%)

* Counts per minute above background in RIA.

† Immunosorbents were prepared using 12 × 75 mm polystyrene tubes filled with etched 2 mm polystyrene balls to increase surface area. Antigens were allowed to coat these in a manner similar to preparation of tubes for the RIA. Aliquots of culture supernatants were absorbed with immobilized antigen and then assayed for specific antibodies by the usual RIA.

§ % control (sample adsorbed with BSA).

Identification of "hidden" or "blocked" anti-Fab' antibodies in 10–19-d-old culture supernatants. The decrease in IgM anti-Fab' activity observed towards the end of many PBL cultures suggested that either these Ab were selectively catabolized or they formed immune complexes with other immunoglobulins. To evaluate the last possibility, we tested selected supernatants for blocked anti-Fab' activity. A modified Farr assay was used in which acidification served to dissociate complexes (11). Supernatants from several cul-

tures that had demonstrated a drop in IgM anti-Fab' activity were tested. Several fold more Fab' was precipitated by 12% PEG from supernatants that had been briefly acidified and then neutralized after the addition of the Ag, as compared with sham-acidified supernatants. In three cases anti-Fab' was demonstrated after acidification in supernatants where none had been detected previously (Table III). Acidification and neutralization of the culture supernatant/¹²⁵I-Fab' mixture resulted in far more Fab' being precipitated

TABLE II
Effect of Addition of IgM Anti-Fc on Measurement of Antibodies to SKSD
and Fab' in Culture Supernatants

Sample	Antibody activity*		
	Anti-Fc	Anti-Fab'	Anti-SKSD
Purified anti-Fc‡	8,489	712	815
Culture supernatant§	16,217	7,357	5,602
Culture supernatant + purified anti-Fc	20,146	7,450	4,926

* Counts per minute above background in the RIA.

‡ Purified anti-Fc was diluted to a concentration typically found in RA patients' cultures. 0.5 ml was mixed with 0.5 ml 1% BSA and assayed.

§ 0.5 ml culture supernatant was mixed with 0.5 ml 1% BSA and assayed.

^{||} 0.5 ml of diluted anti-Fc plus 0.5 ml of culture supernatant were mixed directly in the antigen-coated tubes for the RIA.

than was precipitated in mixtures in which the culture supernatant or ^{125}I -Fab' was acidified by itself and neutralized before mixing with the other component (Table IV). These results suggest that this approach indeed unblocked hidden anti-Fab' Ab.

IgM anti-Fc found after 24 h in culture in supernatants from RA patients is mainly newly synthesized antibody. The high levels of IgM anti-Fc detected after only 24 h in vitro could be explained either by carryover of IgM anti-Fc from the peripheral blood or by early synthesis. To evaluate these alternatives, PBL from four patients whose previous cultures had exhibited early IgM anti-Fc release were cultured again with and without cycloheximide to block protein synthesis. Freeze/thaw controls were used to quantitate the amount of Ab carried over "in" and/or "on" the cells. The results from one of these four is shown in Fig. 3. Cultures from two of the four patients contained more IgM anti-Fc than the freeze/thaw control after 24 h in vitro; cultures from all four exceeded this base line by 48 h. In each case the early release was completely blocked by cycloheximide.

Effect of PWM on IgM anti-Fc synthesis. Cultured PBL from the patient presented in Fig. 3 released more IgM anti-Fc when PWM was omitted. Suppression of IgM anti-Fc synthesis by PWM was seen in 4 of 17 patients. In each case the patient was among those whose PBL produced relatively large quantities of IgM anti-Fc (mean 186 ± 38 ng). In three of the four patients, PWM selectively depressed IgM anti-Fc but enhanced release of anti-Fab' and anti-SKSD (Fig. 4). Lymphocyte cultures from a fourth patient were set up three times over the span of 1 yr. On two occasions only the release of anti-Fc was depressed by PWM stimulation. On one occasion all three Ab were lower after PWM. By contrast, PWM always enhanced the amount of Ab released by cultured PBL from normal donors. The amount of Ab released after PWM stimulation was directly proportional to the amount that was released in the corresponding unstimulated culture, (IgM anti-Fc, $r_s = 0.74$, $P < 0.01$; IgM anti-SKSD, $r_s = 0.74$, $P < 0.01$; Spearman rank correlation test).

Maximum quantities of specific Ab released in vitro. To compare the maximum quantities of Ab produced by PBL from RA patients and healthy donors, levels were measured after 11–18 d of culture (Fig. 5). In the absence of PWM, cultures from RA donors contained 122 ± 106 ng IgM anti-Fc vs. 27 ± 27 for PBL from healthy donors, ($P < 0.001$, Mann-Whitney U test). With PWM, RA patients' PBL released 184 ± 123 ng IgM anti-Fc and normal donors', 165 ± 111 ng, ($P > 0.05$, Mann-Whitney U test). The same PWM-stimulated cultures from RA patients contained 73 ± 43 ng of IgM anti-SKSD, whereas cultured cells from controls

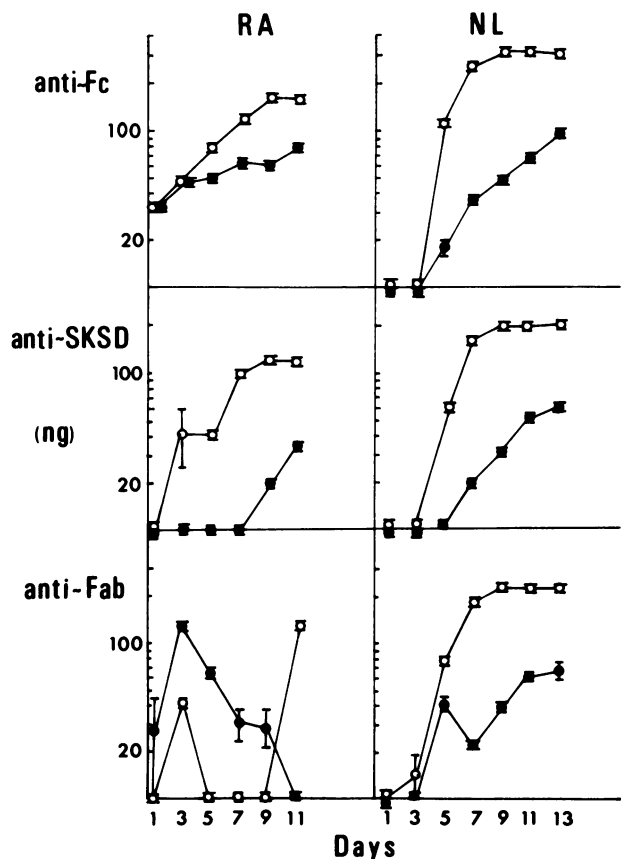


FIGURE 1 Kinetics of specific antibody release by PBL from a representative RA patient (RA) and a normal control (NL). Cells were cultured for 1–13 d with (○) and without (●) 1% PWM. At intervals, cultures were harvested, supernatants were pooled and assayed by the RIA for IgM anti-Fc, anti-SKSD, and anti-Fab'. Results show nanograms bound in 1 ml of sample diluted 1:2. Error bars represent ± 1 SD from the mean of three replicates.

made 99 ± 68 ng, ($P > 0.05$, Mann-Whitney U test). In the absence of PWM, cells from RA patients frequently failed to make detectable amounts of IgM anti-SKSD in vitro. Cultures from 22 of 30 control donors had >10 ng of IgM anti-SKSD, whereas only 4 of 14 RA patients achieved this level, ($0.02 < P < 0.05$, chi-square test).

Relationship of Ab biosynthesis to treatment. Early release of IgM anti-Fc and relatively high levels of this Ab were observed equally in cultures from patients receiving corticosteroids, D-penicillamine, or gold. PBL from two patients were cultured a second time when their therapy had been inadvertently discontinued. Early release and relatively high levels of IgM anti-Fc were still prominent features of the in vitro response of their PBL.

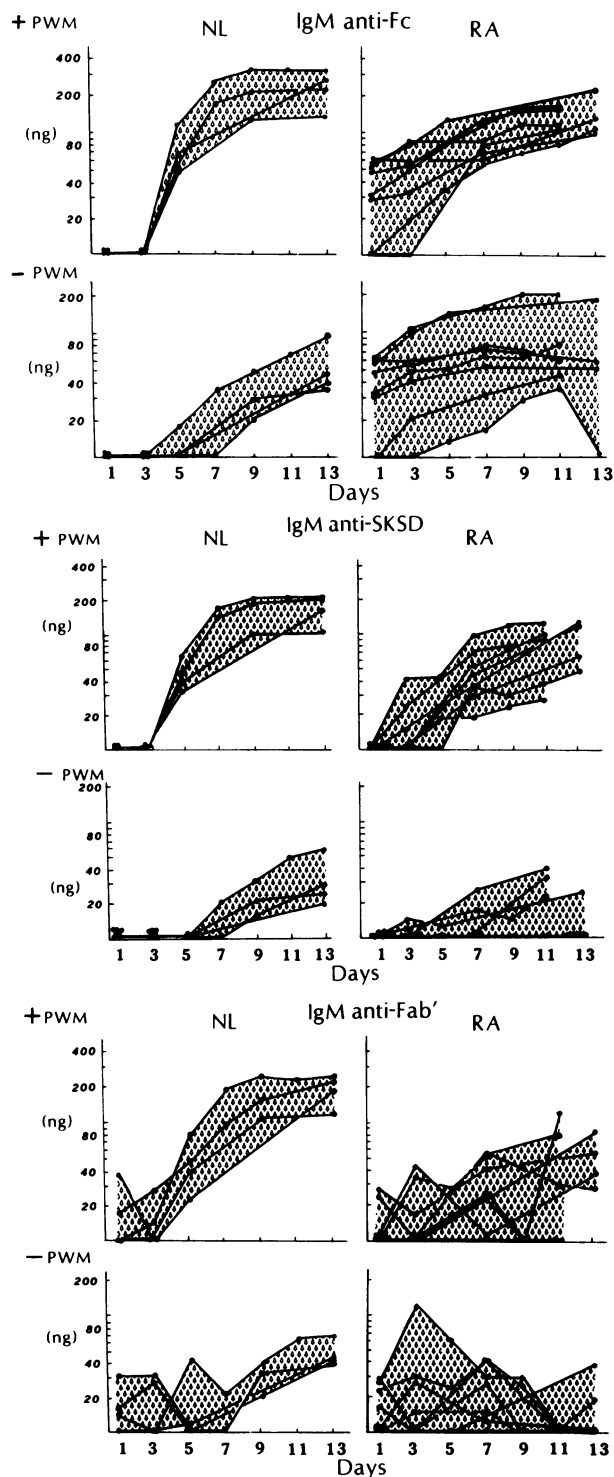


FIGURE 2 Kinetics of specific antibody production in eight RA and four normal donors. Lymphocytes were cultured with and without PWM. Supernatants were harvested at periodic intervals and assayed for: (a) IgM anti-Fc, (b) IgM anti-SKSD, and (c) IgM anti-Fab'. Results show nanograms bound in 1 ml of sample diluted 1:2. Each point represents the mean of three replicates from PBL of one donor, harvested at that time. NL = normal, RA = rheumatoid arthritis.

TABLE III
Precipitation of ^{125}I -Fab' by Anti-Fab' in Culture Supernatants in the Presence of 12% Polyethylene Glycol. Effect of Acidification and Neutralization of the Culture Supernatants

Neurulation and Acidification of the Embryo						Cycloheximide	
Patient no.		With acidification	Without acidification	With acidification	Without acidification	With acidification	Without acidification
		day 10		day 17-19			
A*	PWM	4,532±211†	572±524	2,782±1,550	1,274±402	ND	
	No PWM	4,522±511	551±98	3,301±2,381	2,104±200		
B*	PWM	4,734±654	-250±156	3,411±539	435±221	ND	
	No PWM	1,876±290	-191±546	4,272±270	131±529		
C*	PWM	2,051±1164	487±409	3,010±356	1,572±134	ND	
	No PWM	3,302±581	661±241	3,070±1,186	946±586		
D*	PWM	ND		3,072±227	-161±110	ND	
	No PWM			1,724±535	-96±327		
E*	PWM	ND		3,404±540	-406±649	ND	
	No PWM			4,370±379	539±308		
F‡	PWM	ND		6,488±353	2,061±544	215±548	688±328
	No PWM			5,757±342	1,879±486		
G‡	PWM	ND		5,587±430	2,004±294	2,070±551	303±255
	No PWM			5,842±493	1,519±299		

* RA donor.

† Data shows mean cpm ^{125}I -Fab' of triplicates ±SD in PEG precipitates. The mean counts per minute ^{125}I -Fab' precipitated by 12% PEG in the absence of specific antibody was subtracted from each value before calculating mean and SE of experimental samples. With acidification 13,791±975 cpm of ^{125}I -Fab' were precipitated; without acidification 6718±988 cpm were precipitated.

‡ Normal donor.

Of the four patients with PWM-associated suppression of IgM anti-Fc release, one was receiving steroids, one, gold, and two, D-penicillamine.

DISCUSSION

There were four salient differences in the responses of cultured lymphocytes from RA patients and normal controls. (a) Cells from some patients made comparatively large amounts of IgM anti-Fc without mitogenic stimulation. (b) Lymphocytes from a few actually released less IgM anti-Fc when PWM was added to the cultures. (c) Cells from the majority of patients failed to make detectable quantities of IgM anti-SKSD unless stimulated with PWM. (d) The kinetics of release of IgM anti-Fab', -anti-SKSD as well as -anti-Fc were relatively erratic in samples from RA donors. Thus these studies of cultured PBL suggest that Ab biosynthesis in RA is abnormal in several respects.

Release of large quantities of Ab in the absence of PWM appeared to be restricted to IgM anti-Fc. Neither anti-SKSD nor anti-Fab' were produced in such quantities nor with such accelerated kinetics. Blockade of Ab release during the first few days in culture with cycloheximide suggests that the PBL of some RA patients had been selectively activated in vivo to synthesize IgM anti-Fc. The high levels of IgM anti-Fc release in cultures from RA patients may reflect the transfer of expanded numbers of activated B cells, increased numbers of specific helper cells, and/or decreased numbers or function of cells that regulate antiimmunoglobulin production. The activation of these B cells in RA and other diseases may be influenced by environmental agents and by genetic factors associated with HLA-DRw4 (15, 16), or the homozygous inheritance of certain Gm allotypes (17).

When stimulated with PWM, PBL from normal con-

TABLE IV
Evidence that Acidification-Neutralization Revealed Blocked
Anti-Fab' Antibody

	Patient 1	Patient 2
Supernatant and ^{125}I -Fab', mixed but not acidified.	44 \pm 48*	162 \pm 45
Supernatant and ^{125}I -Fab' mixed before acidification, and neutralization	1897 \pm 245	1669 \pm 641
^{125}I -Fab' acidified and neutralized before adding supernatant	824 \pm 185	620 \pm 160
Supernatant acidified and neutralized before adding ^{125}I -Fab'	349 \pm 82	714 \pm 22

*Mean counts per minute ^{125}I -Fab' of triplicates \pm SD precipitated by 12% PEG.

trols were capable of producing as much IgM anti-Fc as PBL from RA patients. The small quantities of IgM anti-Fc released by normal PBL in the absence of PWM suggests that their PBL had not encountered the appropriate activating stimulus or that synthesis of this Ab was actively suppressed. Koopman (18) has reported preliminary evidence that cells capable of suppressing the IgM anti-Fc response circulate in normal persons.

In RA patients, release of IgM anti-SKSD was demonstrated relatively infrequently in unstimulated PBL, yet after PWM stimulation, PBL from these same individuals produced quantities of IgM anti-SKSD equivalent to those found in supernatants from normal donors. This observation suggests that effective suppression of specific Ab biosynthesis can occur in RA

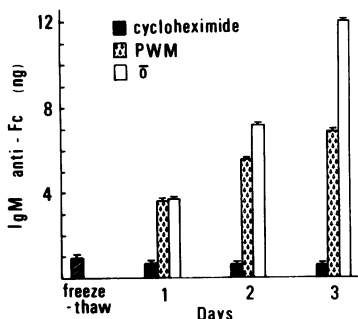


FIGURE 3 Early kinetics of IgM anti-Fc release by one RA donor. Cells were cultured for 1–3 d alone, with 100 $\mu\text{g}/\text{ml}$ cycloheximide, or 1% PWM. Day zero freeze/thaw controls were used to define the amount of antibody carried over into the culture. Results show nanograms bound in a 100- μl test sample. Error bars represent ± 1 SD from the mean of three replicates.

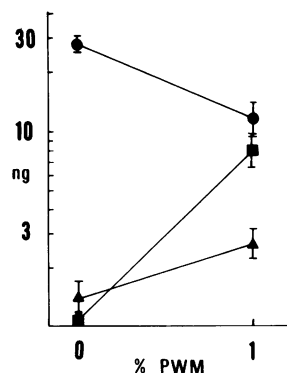


FIGURE 4 Suppressed IgM anti-Fc release in the presence of PWM. PBL from one RA patient were cultured with and without 1% PWM. After 13 d the supernatants were assayed for IgM anti-Fc (●), IgM anti-SKSD (▲), and IgM anti-Fab' (■). Results show nanograms bound in a 100- μl test sample. Error bars represent ± 1 SD from the mean of three replicates.

and is expressed in the regulation of IgM anti-SKSD, and possibly other Ab.

In four RA patients, unstimulated PBL produced more IgM anti-Fc than PBL cultured with PWM. The IgM anti-Fc levels in unstimulated cultures from these four were among the highest observed among the RA patients. Stevens et al. (19) noted that addition of PWM and T cells led to suppression of IgG antitetanus toxoid production by B cells from donors recently primed by booster immunization. Thus, suppressive effects of PWM may only be realized among cell populations that are already activated or engaged in synthesis of specific Ab.

The amount of IgM anti-Fab' in the culture supernatants often fluctuated throughout the culture period. Although these fluctuations were evident in cultures from both normals and RA patients, the widest swings in IgM anti-Fab' were seen in samples from RA donors. In many cases IgM anti-Fc and -anti-SKSD rose at times when the IgM anti-Fab' was falling. Using a modified Farr assay, we found that brief acidification of culture supernatants to dissociate soluble complexes demonstrated considerable quantities of blocked anti-Fab' activity in culture supernatants from both normal donors and RA patients.

The antigenic specificities of the IgM anti-Fab' produced by these cultured PBL have not been defined. Some may be antiidiotypic. Bonagura et al. (20) have reported that ~60% of monoclonal IgM with anti-gamma globulin activity share a cross-reacting idiotype. B cells marked by this cross-reacting idiotype were relatively abundant among PWM-stimulated cultures of PBL from patients with active RA. Thus, clones producing antiidiotypes corresponding to these IgM anti-Fc may also be expanded in RA patients (21).

Similar response patterns were detected in PBL

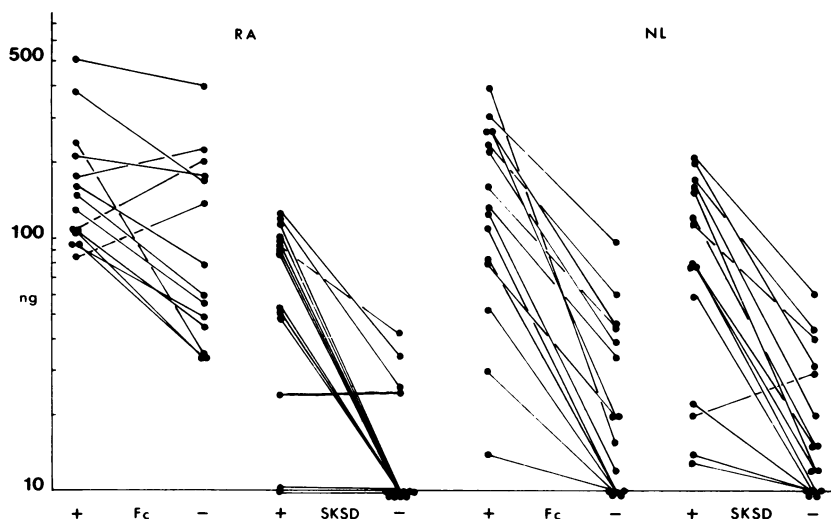


FIGURE 5 Comparison of levels of specific antibody synthesized in vitro. Lymphocytes were cultured with (+) and without (–) PWM. At the end of 11–18 d, the supernatants were assayed for: IgM anti-Fc and IgM anti-SKSD. Results indicate nanograms bound in 1 ml of sample diluted 1:2. Each point represents the mean of three replicates.

from patients on gold salts, D-penicillamine, and corticosteroids. Two individuals, studied while off all therapy, showed the same abnormal responses as measured previously during treatment. Although gold salts and D-penicillamine can modulate Ab biosynthesis by PBL in vitro (22, 23), it is not likely that these drugs, upon administration to patients, contribute to the high levels of IgM anti-Fc and the other specific abnormalities observed in these investigations. When added in vitro, gold salts and D-penicillamine inhibit lymphocyte proliferation and mitogen-induced immunoglobulin production (22–25). Studies of patients before and after treatment and at repeated intervals during both remissions and exacerbations may help to sort out the relative influences of drug and disease on Ab biosynthesis in RA.

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