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HIGH LEVELS OF IN VITRO IgM RHEUMATOID FACTOR SYNTHESIS CORRELATE WITH HLA–DR4 IN NORMAL INDIVIDUALS

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The association between the HLA–DR4 histocompatibility antigen and in vitro synthesis of IgM and IgM rheumatoid factor (IgM-RF) by blood mononuclear cells was investigated in 35 normal subjects. In vitro cultures of T and B cells were stimulated with pokeweed mitogen, and the secreted IgM-RF, tetanus antibody, and total IgM protein were measured by solid-phase radioimmunoassay. In cultures containing unseparated T cells, IgM-RF production in the DR4+ and DR4− subgroups was not significantly different. However, depletion of OKT8+ cells containing T suppressor cells resulted in significantly higher IgM and IgM-RF synthesis in the DR4+ subgroup. Moreover, 6 of the 8 highest levels of IgM-RF were produced by DR4+ individuals, while only 7 of the remaining 27 individuals were DR4+ ($P = 0.035$). Ratios of secreted IgM-RF : IgM indicated that there was a relative enrichment for IgM-RF–specific B cell precursors in DR4+ high responders, although total numbers of circulating B cells were not increased. High responder B cells had high levels of responsiveness when mixed with T helper cells from low responders, whereas low responder B cells consistently produced low responses, even when cocultured with T helper cells from high responder donors. The data suggest that a subset of normal individuals has a pokeweed mitogen–responsive lymphocyte population that contains a B cell subpopulation specific for IgM-RF synthesis and that this condition is associated with HLA–DR4. In certain individuals who have the DR4 type, there may be a component of a susceptible genetic background, upon which other factors act to induce IgM-RF synthesis and which may produce clinical manifestations of rheumatoid arthritis.

Rheumatoid arthritis (RA) is associated with HLA–DR4, a serologically determined genetic marker within the human major histocompatibility complex (1,2). Approximately 65% of RA patients have an HLA–DR4 haplotype; this is significantly higher than the 28% incidence found in a comparable control population. The finding that patients with seronegative RA do not have an increased incidence of HLA–DR4 (3–5) suggests that the gene may exert its effect on the disease by controlling synthesis of rheumatoid factor (RF). While the mechanism by which this might occur is not known, the available evidence suggests that the HLA–D region might influence regulation of the immune response (6–8).

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The development of sensitive solid-phase assays for RF has allowed study of the synthesis of this autoantibody at the cellular level. Using these techniques, it has become clear that a significant percentage of normal individuals possess B cell precursors specific for IgM-RF (9,10). In our studies, a subset of normal individuals was identified in whom in vitro stimulation of blood mononuclear cells (BMC) with pokeweed mitogen (PWM) resulted in high levels of IgM-RF and IgM synthesis. This high responsiveness was found to be correlated with the presence of the HLA–DR4 haplotype. Cell mixing experiments indicated that the B lymphocyte population was responsible for the high responses observed. The data suggest that these DR4+ high responders may also have a preferential expansion of a B cell subpopulation committed to IgM-RF synthesis.

SUBJECTS AND METHODS

Subjects. The study population consisted of 35 normal individuals drawn, for the most part, from students and laboratory personnel. Only 2 donors were more than 40 years of age and none was older than 60. Thirty-two donors were white. None had been diagnosed as having arthritis or other chronic inflammatory diseases. All subjects were completely typed for HLA–A, B, C, DR, and DQ region alleles by microlymphocytotoxicity assay, as previously described (11). The typing reagents used were partly of local origin and partly obtained by exchange with other investigators. The definition of HLA antigens was based on panel typings from the Ninth International Histocompatibility Workshop.

Cell separation. Heparinized peripheral blood was diluted with an equal volume of normal saline and layered over Ficoll-Hypaque cushions as previously described (12). BMC at the interface were washed extensively in Hanks' balanced salt solution and RPMI 1640, both purchased from Gibco (Grand Island, NY). Phagocytic cells were labeled by ingestion of polystyrene particles (Difco, Detroit, MI) at 37°C for 45 minutes. Nonadherent cells were obtained after 2 successive 1-hour incubations on petri dishes and contained less than 5% cells with ingested latex particles. T and B cells were separated by rosetting the nonadherent cells with neuraminidase-treated sheep erythrocytes, followed by centrifugation on Ficoll-Hypaque. T cells were obtained after lysis of the erythrocytes with ammonium chloride. The non–T cell population at the interphase was rosetted a second time to further deplete T cells. The resulting population, referred to as B cells, contained approximately 30% mononuclear phagocytes.

B lymphocytes were quantitated by incubation of washed cells with fluorescein-labeled goat anti-human Ig (high ratio of fluorescein:protein; Meloy, Springfield, VA) at 0°C for 30 minutes. After 3 washes at 4°C, the cell suspensions were examined by ultraviolet microscopy using a Leitz Wetzlar microscope fitted with an epiillumination system. Surface immunoglobulin–positive cells were expressed as a percentage of BMC.

Total T cell populations were depleted of the suppressor:cytotoxic subpopulations defined by the monoclonal antibody OKT8 (Ortho Diagnostics, Raritan, NJ) by negative selection using the panning technique (13). Plastic 35-mm petri dishes (Falcon #3001; Becton Dickinson, Oxnard, CA) were coated with 40 μg/ml affinity-purified goat anti-mouse Ig (the kind gift of Dr. Ellen Vitetta). Prior to use, pans were washed with phosphate buffered saline (PBS) containing 1% fetal calf serum (FCS). Ten million T cells were labeled with 10 μl of OKT8 antibody at 0°C for 30 minutes, washed, diluted to 1 ml in PBS–1% FCS, and added to prepared pans for 60 minutes at room temperature. The nonadherent population was gently aspirated from the pan, washed, and resuspended in culture medium. This one-step panning procedure routinely depleted OKT8+ cells to <4%, while enriching the OKT4+ population to >80% of the cells.

Cell cultures. Cultures were performed in 96-well, round-bottom microtiter plates (Dynatech, Alexandria, VA). Cell populations were suspended in RPMI 1640 containing 10% FCS, glutamine, penicillin, and gentamicin. Each microwell contained 50,000 B cells and 25,000–100,000 T cells in a total volume of 0.20 ml. Five replicate cultures were done with and without the addition of PWM (Gibco) at final concentrations of 1:250 or 1:500. After a 13-day incubation period, the microtiter plates were centrifuged at 600 revolutions per minute and the replicate cell-free supernatants were pooled. The supernatants were stored at −20°C until assayed.

Radioimmunoassays. The total IgM secreted in culture supernatants was measured by solid-phase radioimmunoassay as previously described (12). Briefly, flexible polyvinyl chloride microtiter plates (Dynatech) were coated with affinity-purified rabbit anti-human IgM. This was followed by washing with PBS and with PBS containing 10% newborn calf serum. Culture supernatants or diluted plasma samples were added at 50 μl/well and were incubated overnight at room temperature. After another washing cycle, ^{125}I-labeled F(ab')$_2$ fragment of the anti-IgM was added to each well. Following another overnight incubation and washing, the wells were cut apart and bound radioactivity was measured in a gamma scintillation counter. The concentration of IgM was calculated using a curve derived from standardized, pooled normal human serum. This assay is sensitive to 25 ng/ml of secreted IgM.

Secreted RF was measured in a similar assay using 50 μg/ml heat-aggregated human IgG as the first coat. Values were normalized by including 5 seropositive RA plasmas in each assay. Results were expressed as nanograms of anti-IgM bound per well. Plasma RF was measured using 1:1,000 dilutions. The upper limit of normal was 2,500 ng of anti-IgM (mean ± 2 SD).

Tetanus IgM antibodies were measured in a similar way. In this case, purified tetanus toxoid (60 μg/ml; Lederle, Pearl River, NY) was added as the first coat. Results were expressed as nanograms per milliliter of secreted IgM anti-tetanus.

IgG concentrations were measured in selected supernatants using a similar assay. Affinity-purified anti-human IgG was bound to the microtiter plates, and ^{125}I-labeled F(ab')$_2$ anti-IgG was used as the developing antibody. The

supernatants were diluted in PBS–10% newborn calf serum containing 0.05*M* dithiothreitol to avoid artifacts created by the presence of RF in the culture supernatants, which could bind IgG nonspecifically.

Statistical analysis. Significance was analyzed using the Mann-Whitney U test and the Wilcoxon (matched pairs) signed rank test. The double-entry contingency table was analyzed by Fisher's exact test. P values <0.05 were considered significant.

RESULTS

In baseline cultures containing unseparated T cell populations, there was no significant difference between the DR4+ and DR4− individuals in either total IgM or IgM-RF secretion (Table 1). However, when the T cell populations were depleted of T8+ cells by panning, significant differences emerged. As shown in Table 1, 13 DR4+ donors had a mean ± SEM IgM-RF secretion of 13.7 ± 3.6 ng of anti-IgM. This was significantly higher than the corresponding value of 5.3 ± 1.4 in 22 DR4− donors ($P < 0.025$). The high response was not limited to RF, however. The mean IgM of 25,220 ng/ml in the DR4+ group was also significantly higher than the 14,100 ng/ml produced by DR4− subjects.

Of additional interest is the distribution of responses in the DR4 positive and negative subgroups. As shown in Figure 1, the secreted RF in DR4+ donors ranged from 0 to >20 ng of anti-IgM. When an arbitrary value of >12 ng of anti-IgM bound was used to define RF high responders, it is apparent that 6 of 13 DR4+ donors were high responders, while only 2 of 22 DR4− donors were high responders ($P < 0.032$).

Stability of high or low producer phenotypes was observed in 25 of the 35 individuals studied who were tested at least twice. Many of these individuals were evaluated several times over a 3-year period. For example, 1 high responder had RF determinations made on 4 occasions with the following results: 29.0, 45.6, 44.3, and 29.5 ng of anti-IgM. Another high responder had RF levels of 32.4 and 24.7 ng of anti-IgM in 2 separate experiments.

Analysis of the distribution of total IgM responses indicated that a positive correlation of high response with DR4 was not limited to RF. As shown in Figure 1, the 4 donors who produced more than 40,000 ng/ml of IgM were DR4+. In contrast, only 2 of 12

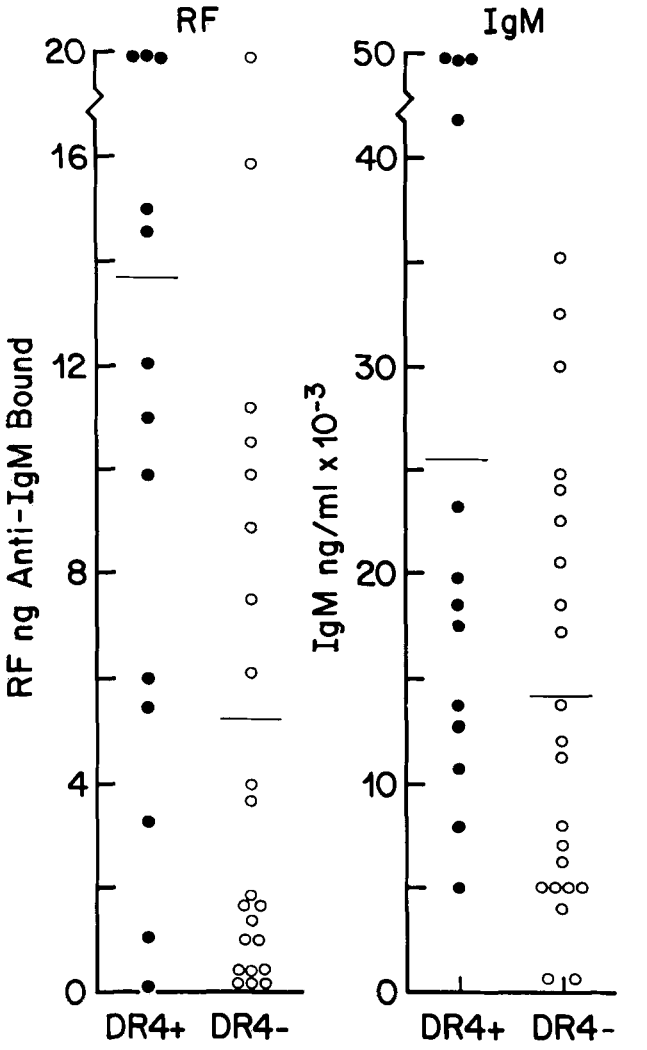

Figure 1. IgM rheumatoid factor (RF) and IgM synthesis by in vitro cultures of B cells and T8-depleted T cells from 35 normal subjects. Horizontal lines indicate the means for each group.

Table 1. IgM and IgM rheumatoid factor (IgM-RF) synthesis in cell cultures from normal individuals*

Donor group	Baseline		T8-depleted	
	IgM-RF (ng anti-IgM)	IgM (ng/ml)	IgM-RF (ng anti-IgM)	IgM (ng/ml)
DR4+ (n = 13)	8.3 ± 3.9†	13,760 ± 4,050†	13.7 ± 3.6‡	25,220 ± 4,910§
DR4− (n = 22)	3.8 ± 1.3	8,540 ± 1,670	5.3 ± 1.4	14,100 ± 2,225

* Cultures contained 50,000 B cells + 50,000 autologous unseparated T cells (baseline), or 50,000 autologous T8-depleted T cells. Values represent mean ± SEM.
† $P > 0.10$ versus DR4− group.
‡ $P < 0.025$ versus DR4− group.
§ $P < 0.05$ versus DR4− group.

donors who produced less than 10,000 ng/ml were DR4+ ($\chi^2 = 4.91$, $P = 0.025$).

In general, levels of synthesized IgM-RF and total IgM were correlated (r = 0.41, $P = 0.014$). However, there was a tendency for DR4 positivity to be more closely correlated with high levels of IgM-RF than with total IgM. For example, while 6 of the 8 highest IgM-RF levels were from DR4+ donors, only 4 of the 8 highest IgM levels were from this group. An additional indication of the relative specificity for RF is obtained by analysis of IgM-RF:IgM ratios. For the 8 RF high responders, the mean ± SEM IgM-RF:IgM ratio in T8-depleted cultures was 1.04 ± 0.21, while for 24 low responder donors with measurable IgM-RF, this value was 0.35 ± 0.18 ($P < 0.025$). Moreover, the tetanus antibody:IgM ratios measured in the same supernatants were 10.6 ± 1.6 and 6.5 ± 1.3, respectively ($P > 0.1$). This suggests that increased IgM-RF synthesis in the DR4+ high responders was the result of preferential expansion or activation of cell populations specific for RF.

To rule out the possibility that the high IgM-RF values obtained were caused by nonspecific binding of high concentratins of IgM to the plastic wells used for the radioimmunoassays, supernatants containing large amounts of IgM and IgM-RF were absorbed 3 times on plastic wells coated with aggregated IgG or on control wells. As shown in Table 2, absorption on aggregated IgG–coated wells resulted in significant decreases in IgM-RF, whereas total IgM remained unchanged.

Total IgG was also measured in 13 supernatants from T8-depleted cultures. A close correlation with total IgM was demonstrated (r = 0.84, $P = 0.003$), indicating that high responsiveness was not limited to the IgM class but was also extended to IgG.

In addition to PWM-induced RF synthesis, plasma IgM-RF levels were measured in 32 subjects. Seven high responders had a mean plasma IgM-RF level of 1,165 ng anti-IgM, while 25 DR4− donors had a corresponding value of 1,059 ng anti-IgM. Mean plasma IgM levels in the 2 groups were similar (1,125 ng/ml and 1,120 ng/ml, respectively). Plasma IgM-RF values were within the normal range in 31 of the 32 subjects.

Table 2. Specific absorption of IgM rheumatoid factor (IgM-RF) by aggregated IgG*

	Control		Aggregated IgG	
Supernatant	IgM (ng/ml)	IgM-RF (ng anti-IgM)	IgM (ng/ml)	IgM-RF (ng anti-IgM)
1	20,000	36.4	17,500	4.7
2	13,500	61.0	14,500	16.0
3	24,500	50.3	27,500	9.3
4	32,000	15.9	34,000	10.8

* Culture supernatants were serially absorbed 3 times on microtiter wells coated with aggregated IgG or on control wells.

Table 3. HLA phenotypes of 8 normal subjects who produced high levels of IgM rheumatoid factor in vitro

	HLA locus				
Donor	A	B	C	DR	DQ
1	1,2	35	w3,w4	4,–,w53	w3,–
2	28,w31	w51,w57	w6	4,–,w53	w3,–
3	w30	w38,w52	—	2,4,w53	w1,w3
4	1,2	37,w62	w3	2,3,w52	w1,w2
5	NT*	NT	NT	3,4,w52,w53	w2,w3
6	2,26	5,18	—	4,–,w53	w3,–
7	2,28	7,60	w3,–	4,–,w53	w3,–
8	2,–	35,44	w4,–	w6,7,w52,w53	w1,w2

* NT = not tested.

The complete HLA phenotypes for the 8 IgM-RF high responders (>12 ng anti-IgM bound) are shown in Table 3. DR4 was the only specificity which was correlated with high responsiveness for RF ($P = 0.035$).

Further studies were designed to investigate the underlying cellular mechanisms responsible for high responsiveness. One possible explanation was that high responders had more circulating B cells. In 10 normal low responders, B cells identified with fluoresceinated anti-Ig constituted 8.1 ± 1.2% of blood lymphocytes (mean ± SEM), whereas 3 high responders had values of 6.0%, 6.6%, and 8.0%. Thus, it is unlikely that an increased number of circulating B cells was present in the high responders. Another possibility was that the procedure by which B cells were isolated may have resulted in higher B cell yields in the high responder individuals. However, quantitation of B cells in purified fractions from 4 high responders demonstrated no increase in B cell percentage compared with that in 4 low responders (data not shown).

It was then necessary to evaluate whether low responses in the low responder cultures were due to decreased sensitivity of B cells to the added T helper cells. If this were the case, it might be possible to induce high responsiveness simply by increasing numbers of T helper cells. In most experiments, cultures contained 50,000 B cells and 50,000 T cells. This ratio was chosen on the basis of our previous studies, which demonstrated this to be optimum for normal PWM-driven antibody synthesis (14). To further investigate

Table 4. Effect of addition of graded numbers of autologous T4+ helper cells on total IgM and IgM rheumatoid factor (IgM-RF) synthesis in low and high producer subgroups

Donor phenotype	Number of T8-depleted cells added*					
	25,000		50,000		100,000	
	IgM-RF	IgM	IgM-RF	IgM	IgM-RF	IgM
DR4− (n = 12)	2.7 ± 0.6	15,000 ± 6,300	1.9 ± 0.7	13,700 ± 3,700	4.4 ± 1.2	16,000 ± 6,350
DR4+ (n = 4)	25.9 ± 8.0	25,800 ± 8,600	21.6 ± 8.0	24,000 ± 9,050	ND	ND

* All cultures contained 50,000 B cells. Values represent mean ± SEM. ND = not done.

these influences, experiments were performed with graded numbers of T cells, from 25,000 to 100,000 per culture. As shown in Table 4, mean values from 12 DR4− low responders studied showed some increase in amounts of synthesized IgM and IgM-RF with increasing T cell numbers, but these changes were not statistically significant ($P > 0.1$). More importantly, with the addition of 100,000 T helper cells, low responder B cells synthesized less than 20% of the mean amount of IgM-RF produced by high responder cultures containing only 25,000 T helper cells (Table 4). Thus, it appears unlikely that high responsiveness for RF could be achieved in low responder donors by adding increased numbers of T helper cells.

The observation that high responsiveness was revealed only in T8-depleted cultures suggested a central regulatory role for T cells, as has been described to explain differences in PWM responsiveness in normal populations (15,16). To analyze this possibility, individual baseline cultures containing total T cell populations were compared with the corresponding T8-depleted cultures in 24 low responders and 6 high responders, from whom paired cultures were available (Figure 2). In the low responder group, mean ± SEM IgM-RF synthesis increased slightly from 2.5 ± 0.7 to 3.2 ± 0.7 ng of anti-IgM ($P < 0.05$), while IgM synthesis showed an increase from 8,030 ± 1,640 to 13,034 ± 2,060 ng/ml ($P < 0.003$). In corresponding cultures from the 6 high responders, IgM increased from a baseline of 17,370 ± 4,365 to 23,950 ± 5,370 ng/ml in T8-depleted cultures ($P > 0.25$), while IgM-RF synthesis almost doubled, from 15.0 ± 5.0 to 24.6 ± 5.3 ng of anti-IgM ($P < 0.05$).

This analysis can be extended by comparing IgM-RF:IgM ratios in the same paired cultures. In 24 low responders, the mean ± SEM baseline IgM-RF:IgM ratio of 0.54 ± 0.15 decreased to 0.35 ± 0.18 in the T8-depleted cultures. In contrast, the corresponding IgM-RF:IgM ratio in 6 high responders increased from 0.93 ± 0.18 to 1.18 ± 0.24 ($P < 0.025$, high versus low responder T8-depleted cultures). Since total IgM increased to a similar extent in both groups, the most likely explanation for these data is that high responder B cell populations were relatively enriched for IgM-RF precursor cells, which were partially suppressed by the T8+ population. Removal of these suppressor cells resulted in a more complete display of the B cell repertoire.

To further investigate the relative contributions of T and B lymphocytes to the high responsiveness, cell-mixing experiments were performed. In each experiment, cell subpopulations from both a low and a

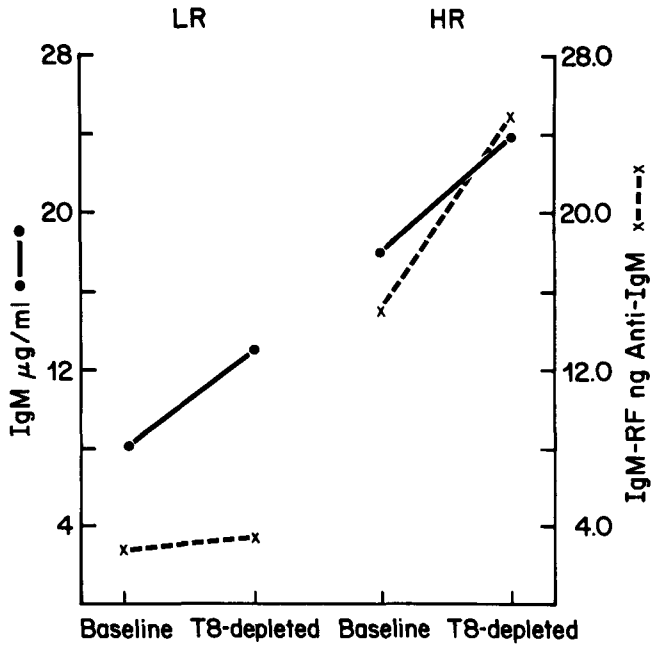

Figure 2. IgM rheumatoid factor (IgM-RF) and IgM synthesis in T8-depleted cultures compared with paired control cultures containing total T cells. Values represent the means for 24 low responder (LR) and 6 high responder (HR) subjects.

Table 5. IgM rheumatoid factor (IgM-RF) and total IgM synthesis in cocultures of T cells and B cells from low and high responder normal individuals*

Cell source†		IgM-RF	IgM	
T	B	(ng anti-IgM)	(ng/ml)	IgM-RF:IgM
HR	HR	22.7 ± 11.6	20,000 ± 5,140	1.1
LR	HR	18.1 ± 5.6	19,500 ± 6,940	0.9
LR	LR	2.4 ± 1.6	7,250 ± 1,100	0.3
HR	LR	2.7 ± 0.2	5,030 ± 2,160	0.5

* Each culture contained 50,000 B cells and 50,000 T8-depleted T cells. Values represent mean ± SEM from experiments using 3 separate donors.
† HR = high responder (DR4+); LR = low responder (DR4–).

high responder were mixed and cultured in the presence of PWM. The mean values from 3 parallel experiments in which high or low responder B cells were paired with high or low responder T cells are shown in Table 5. The results clearly demonstrate that high responsiveness for IgM-RF synthesis resided with high responder B cells, even when the T helper population was from a low responder donor. In addition, cultures containing high responder B cells were relatively enriched for IgM-RF synthesis, as shown by the higher IgM-RF:IgM ratios. The source of the T cells did not appear to affect this ratio significantly. These results provide further evidence to suggest that an expanded B cell subpopulation in high responders was responsible for the production of IgM-RF.

DISCUSSION

In this study, 8 of 35 normal individuals were found to have increased PWM-induced in vitro synthesis of IgM-RF and IgM in cultures depeleted of T8+ T suppressor cells. Six of 8 high responders were DR4+, and although the separation of high and low responder groups was made on the basis of arbitrary levels of synthesis, the overall mean value for IgM-RF synthesis in 13 DR4+ normal donors was significantly greater than that in 22 DR4− individuals. Total IgM synthesis was also increased, but the relative increase in IgM-RF synthesis was greater. Investigation of the cellular mechanisms involved indicated that there was an increase in the PWM-responsive B cell population in the high responder donors. Thus, in the absence of suppressor influences, polyclonal activation of circulating B lymphocytes revealed the presence of expanded cell subpopulations specific for RF synthesis within a PWM-responsive cell population in a subset of normal donors.

Although the B cell populations used in this study contained significant numbers of monocytes and some natural killer (NK) cells, it is unlikely that the differences in IgM-RF and IgM synthesis were caused by the contaminating cells. Experiments in which stringent depletion of monocytes and NK cells was accomplished by incubation with L-leucine-methyl ester (17) prior to culture showed no difference in IgM-RF response when compared with that in controls (Jasin HE: unpublished observations).

An association between HLA–Dw4 and HLA–DR4 and RA was initially observed in our clinic (1,2) and has been confirmed in various study populations (3,4,18). However, the presence of HLA–DR4 alone is clearly not sufficient for the development of RA. In most series, only 65–70% of RA patients are found to be DR4+ (2); most DR4+ individuals do not develop the disease. Thus, it appears that while DR4 may be a component of a susceptible background, other genetic and/or environmental factors are necessary for the development of disease.

The mechanism by which HLA genotypes control susceptibility or clinical manifestations in RA or other diseases is unknown. One clue in RA is that the relationship with DR4 is most firmly established for seropositive, as opposed to seronegative disease (3). This suggests that the mechanism by which DR4 predisposes an individual to the development of RA involves control of IgM-RF synthesis. With the availability of sensitive radioimmunoassays for RF, it has become clear that RF precursors can be detected in at least 50% of normal individuals (9,10). From this observation, it follows that the capability to synthesize IgM-RF is not in itself pathogenic. However, circulating cells that spontaneously synthesize this autoantibody are seen only in RA patients (9,12). Thus, it may be that HLA–DR4 or a closely linked gene has its effect at the level of control of RF-specific B cell activation.

Of additional interest in this regard is a recent report demonstrating high responsiveness to *Mycobacterium tuberculosis* antigens in DR4+ leprosy patients (19). These data suggest that the DR4 locus may control antigen-specific immune responses, which may then be linked to production of RF. The mechanism by which this control is exerted could involve quantitative or qualitative alterations in T or B cells. The results of the current study suggest that high producers of RF, most of whom were DR4+, had a preferential

expansion of the B cell subpopulation committed to IgM-RF synthesis. This was indicated by the significantly higher IgM-RF:IgM ratios of secreted antibody when compared with tetanus antibody:IgM ratios following polyclonal activation in vitro. In addition, the mixing experiments with T and B cells from low and high responders demonstrated that the B cells, not the T helper cells, were responsible for the high responsiveness.

Other studies have suggested that low responsiveness for PWM-induced Ig synthesis in a subset of normal individuals is due to altered regulation at the T cell level (15,16). However, this mechanism does not apply in the present study, since removal of T8+ cells resulted in significant increases in IgM synthesis in both low and high responders. The critical difference in the high responders in our study was that elimination of suppressor cells revealed the presence of an additional subpopulation of PWM-responsive B cells which are normally under the control of the T suppressor cell population (Figure 2).

Preferential expansion of the RF-committed B cell subpopulation could occur in several ways. First, these individuals may have an increase in the intrinsic responsiveness of these B cells to environmental stimuli. Exposure to infectious agents, for example, might result in a high level of stimulation and permanent expansion of an RF-producing B cell subset. Alternatively, the high responders may have a B cell subpopulation that differentiates in vitro into cells that are able to secrete higher levels of IgM and IgM-RF. Another possibility is that high responder B cells may be more responsive to T cell signals that trigger maturation and proliferation (20). On the other hand, the B cells may be intrinsically normal, while the T cells are hyperresponsive to environmental stimuli. These are not necessarily mutually exclusive mechanisms, for it is also possible that the genetic trait could affect both B and T cells in a similar manner. It is not possible to choose between these various possibilities on the basis of the current data. However, the mechanisms posited above lend themselves to empirical verification.

The possible link between in vitro RF synthesis in normal individuals and the presence of the HLA–DR4 genotype has been examined in 2 previous studies. No significant correlation was found in either (21,22). A major reason for the different results obtained in the current study is the use of T suppressor cell–depleted cultures. Cultures containing unseparated total mononuclear cells, as used in both of the previous reports, failed to demonstrate the differences. It is of interest, however, that the study by Rodriguez et al (22) noted a tendency for DR4+ donors to produce RF with Epstein-Barr virus (EBV) activation of B cells more frequently than did DR4− donors. In addition, with weekly followup for 4 weeks, the DR4+ subgroup showed a steadily increasing RF:IgM ratio, while in the DR4− group, this ratio did not increase. These differences were not statistically significant, possibly because the presence of T cell populations in these EBV cultures could limit full expression of antibody synthesis (23). Nevertheless, since EBV is a T-independent B cell activator, the data are consistent with results in this study which suggest that the DR4+ high responders have an expansion of circulating RF-specific precursor B cells.

Further studies of the mechanisms involved in the regulation of RF synthesis in healthy donors and patients may clarify the relationship between the presence of this autoantibody and the immunogenetic factors that determine susceptibility to and lead to the development of RA.

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