PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 168, 344–349 (1981)

Suppressor T Cells Prevent *in Vitro* Expression of IgM Rheumatoid Factor in Some Healthy Adults[1] (41284)

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Abstract. Peripheral blood mononuclear leukocytes (MNL) from 11 of 30 healthy adults elaborated detectable IgM RF when stimulated with pokeweed mitogen. In order to assess the possibility that suppressor T-cell activity might account for the nonexpression of IgM RF *in vitro* in some individuals, the influence of T cells on IgM RF production by autologous B cells prepared from donors whose unfractionated MNL synthesized IgM RF in response to PWM was investigated. Untreated T cells supported IgM RF production by autologous B cells with optimal synthesis observed at T:B cell ratios of 2:1; at higher T:B cell ratios a decline in IgM RF production occurred. In contrast, at higher T:B cell ratios irradiated T cells supported consistently higher levels of IgM RF production than untreated T cells suggesting the presence of radiosensitive suppressor T cells for IgM RF in these individuals. To determine the frequency of these suppressor cells, irradiated T cells were compared to untreated T cells for capacity to support IgM RF production by autologous B cells from 12 randomly selected donors at T:B cell ratios of 3:1. Untreated T cells from 4 of 12 individuals were capable of cooperating in induction of IgM RF production by autologous B cells, whereas irradiated T cells supported IgM RF production in 6 of 12 individuals. Levels of IgM RF production in all 6 individuals were significantly higher with irradiated T cells than with untreated T cells; in 2 individuals IgM RF synthesis by autologous B cells was observed only in the presence of irradiated T cells. In 4 of 6 individuals increases in the ratio of IgM RF total IgM synthesis occurred with irradiated T cells (when compared to untreated T cells), suggesting disproportionate suppression of RF production. These results indicate the presence of radiosensitive T cells capable of suppressing IgM RF production in a significant fraction of healthy adults and raise the possibility that these cells may regulate *in vivo* expression of RF.

Considerable evidence suggests that rheumatoid factors (RF)[2] contribute to the pathogenesis of inflammation in rheumatoid arthritis (RA) (1, 2). For instance, RF have been demonstrated to be a constituent of immune complexes present in the serum (3), synovial fluid (4), and synovial fluid PMNs (5) of patients with RA.

Cellular mechanisms underlying expression of RF are incompletely understood. In our previous studies, circulating B cells capable of synthesizing IgM RF *in vitro* were shown to be present in approximately 40% of healthy adults (6, 7). However, the absence of detectible serum IgM RF in these individuals suggested that activation of B cells programmed to make RF was subject to regulatory influences. In view of evidence that *in vitro* synthesis of IgM RF was T-cell dependent (6–8), the possibility that suppressor mechanisms might contribute to regulation of RF expression seemed plausible. The data in this report indicate that circulating radiosensitive T cells capable of diminishing *in vitro* IgM RF synthesis are demonstrable in some healthy adults.

Materials and Methods. *Preparation and culture of peripheral blood mononuclear leukocytes (MNL).* Peripheral blood was obtained from 42 healthy adult volun-

[1] Supported by NIH Grants AI-547430, AM-03555, and CA-13148.

[2] Abbreviations used: RF, rheumatoid factor(s); RA, rheumatoid arthritis; PMN, polymorphonuclear leukocyte; MNL, mononuclear leukocytes; PWM, pokeweed mitogen; AET, 2-aminoethylisothiouronium bromide; SRBC, sheep red blood cells; VBS-BSA, veronal-buffered saline containing 1% bovine serum albumin.

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teers without personal or family history of rheumatic disease. MNL were prepared from each individual by density centrifugation using the method of Böyum (9). The cells were washed two times in MEM, five times in RPMI 1640 containing 5% heat-inactivated fetal calf serum (Microbiological Associates, Walkersville, Md.), and two times in complete medium (RPMI 1640 containing 10% heat-inactivated fetal calf serum, 2 m*M* glutamine and 20 μg/ml gentamycin). MNL were adjusted to 1×10^6 cells/ml and cultured in 1-ml aliquots in plastic tubes for 7 days at 37° in a 5% CO_2 and air atmosphere. Pokeweed mitogen (PWM) (Grand Island Biological Co., Grand Island, N.Y.) was added to one-half of the cultures (at least quadruplicates) at the initiation of culture in a concentration predetermined to be optimal in our hands (10 μg per culture). At the termination of culture, the tubes were centrifuged and supernatants harvested and frozen at −20° until assayed for IgM and IgM RF.

Isolation of T-cell and enriched B-cell fractions. T cells and enriched B cells were isolated from MNL using the method of Saxon *et al.* (10). Briefly, MNL were rosetted with AET-treated sheep red blood cells (SRBC) at 37° for 10 min and then at 4° for 20–30 min following centrifugation (1000 rpm × 10 min). The mixture was gently layered on Ficoll–Hypaque (Pharmacia Fine Chemicals Inc., Piscataway, N.J.) and the rosetted cells separated from nonrosetting cells by density centrifugation. Both rosetting and nonrosetting fractions were then rerosetted followed by an additional density gradient centrifugation over Ficoll–Hypaque to obtain further purification. SRBC in the T-cell fraction were lysed with a $KHCO_3$-buffered NH_4CL solution. The rosetting population (T cells) consisted of less than 3% nonrosetting cells and less than 1% surface Ig-positive cells (11). The B cell-enriched population consisted of 45–60% surface Ig positive cells and less than 2% rosetting cells.

Irradiation of T cells. In some experiments T lymphocytes (1×10^6 cells/ml in RPMI 1640, containing 5% fetal calf serum) were irradiated (3000 R) with a Pickard–Vanguard Ortho Voltage X-Ray Therapy Unit using 280 kVp at 20 mA with a half-value layer of 1.3 mm of copper at a distance of 38 cm. Doses were delivered at a rate of 136 rad/min. Irradiated T cells (and parallel nonirradiated T cells) were washed twice in complete medium and cell viability determined. No effect on cell viability (trypan blue exclusion) was observed 1 hr after treatment.

Radioimmunoassay of IgM. The method for radioimmunoassy of IgM utilized in this laboratory has been previously described in detail (6). Briefly, dilutions of an isolated monoclonal IgM standard (encompassing the range 0.5 to 15 ng) or of unknown samples were incubated together with 1 ng of ^{125}I-labeled IgM and a dilution of specific goat anti-human IgM such that approximately 50% of the label was precipitated when incubated alone. After 16 hr at room temperature, excess formalinized Cowan I strain *Staphylococcus aureus* was added to each tube and the mixture agitated and incubated an additional hour at room temperature. The tubes were then centrifuged and an aliquot from each tube counted in a gamma counter (Biogamma 1500, Beckman Instruments Inc., Palo Alto, Calif.) Standard curves were constructed for each experiment by plotting mean counts per minute of ^{125}I-labeled IgM in the supernatant for each input of IgM standard. Quantitative values for IgM in unknown samples were calculated by determining the mean counts per minute of ^{125}I-labeled IgM in the supernatant for each dilution of unknown and then by referring to the IgM standard curve. All assays were performed in duplicate or triplicate. Preliminary experiments were conducted which established that IgM RF did not interfere with IgM determinations in the assay.

Radioimmunoassay of IgM rheumatoid factor. The procedure utilized in these studies has been previously reported in detail (12). Briefly, duplicate aliquots of an isolated monoclonal IgM RF (encompassing the dose range 0.5 to 10 ng per tube) and unknown samples were placed in both human IgG and bovine serum albumin (BSA)-coated polypropylene tubes and in-

cubated at room temperature for 18–20 hr. The tubes were then aspirated, washed with 0.15 M NaCl, 0.005 M veronal buffer, pH 7.5, containing 1% radioimmunoassay grade bovine serum albumin (VBS–BSA) and incubated with 10 ng of ^{125}I-labeled anti-human IgM for 4 hr at room temperature. Each tube was aspirated, washed with VBS–BSA, and counted in a gamma counter. Standard curves were constructed for each experiment by plotting mean counts per minute of ^{125}I-labeled anti-IgM bound per IgG-coated tube (minus BSA control) for each input of IgM RF. Quantitative values for unknowns were calculated by determining the mean counts per minute of ^{125}I-labeled anti-IgM bound (IgG tube mean minus BSA tube mean) for each dilution of unknown and then by referring to the RF standard curve. All assays were performed in duplicate. The coefficient of variation for this assay has previously been shown to be 8.2–18.6% depending on the concentration of IgM RF being measured (12).

Statistical methods. Statistical comparisons between group means were made utilizing Student's *t* test and *P* values derived from standard tables by extrapolation.

Results. *In vitro production of IgM rheumatoid factor by MNL from healthy adults.* Peripheral blood MNL from 30 healthy adults were cultured for 7 days in the presence and absence of pokeweed mitogen (PWM). As shown in Fig. 1, IgM RF was not detectable in culture supernatants of MNL cultured in the absence of PWM. In contrast, MNL from 11 of 30 individuals elaborated IgM RF when cultured in the presence of PWM (mean ± 1 SD = 14.7 ± 14.8 ng/10^6 cells). Thus, under these conditions 37% of healthy adults had demonstrable circulating B cells capable of synthesizing IgM RF when stimulated with PWM. The inability to demonstrate RF production by MNL from approximately two-thirds of healthy adults raised the possibility that T-cell influences might contribute to nonexpression of RF in these individuals. In this regard, we have previously shown that PWM-induced synthesis of IgM RF is strictly T-cell dependent (6, 7).

Effect of varying T-cell input on in vitro

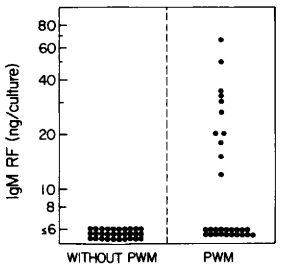

FIG. 1. IgM RF synthesis by cultured peripheral blood MNL from healthy adults. MNL from 30 healthy adult donors were incubated for 7 days in the presence and absence of PWM and culture supernatants then assayed for IgM RF. Quadruplicate cultures were pooled and assayed in duplicate at two dilutions.

IgM RF synthesis by autologous B cells. In order to assess T-cell influence on IgM RF production by B cells, T- and enriched B-cell fractions were initially prepared from healthy donors whose unfractionated MNL synthesized RF in response to PWM. Neither T cells alone nor T cell-depleted B cells synthesized detectable IgM RF in response to PWM (data not shown). Addition of untreated T cells supported PWM-induced RF production by autologous B cells; optimal RF synthesis was generally observed at T:B cell ratios of 2:1 (Figs. 2A and B). At higher T:B cell ratios, suboptimal RF production occurred (Figs. 2A and B).

The possibility that T-suppressor cells might account for the decline in RF production observed at higher T:B cell ratios was investigated by comparing the effect of irradiated versus untreated T cells on RF production by autologous B cells. In this regard, several investigators have previously shown irradiation to be a simple method of selectively eliminating suppressor T-cell activity (13–15). As shown in Figs. 2A and B, irradiated T cells also supported RF production by autologous B cells. At lower T:B cell ratios (up to 2:1), irradiated T cells either induced similar levels of RF synthesis to that observed with untreated T cells (Fig. 2A) or, in some individuals, supported consistently greater RF production by autologous B cells than untreated T cells (Fig. 2B). At higher T:B ratios, further increases in RF production

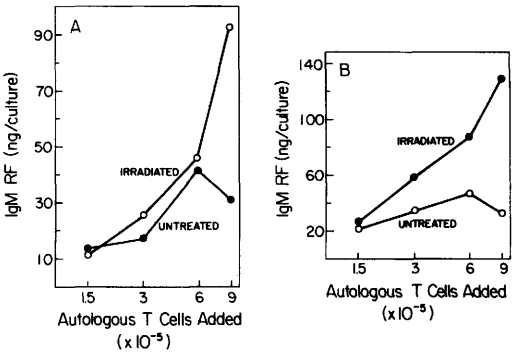

FIG. 2. Dose–response effect of added T cells (untreated versus irradiated) on IgM RF synthesis by autologous B cells. MNL from two representative healthy adult donors (A and B) were separated into T- and enriched B-cell fractions. B cells were cultured at a density of 3×10^5 cells/ml and varying concentrations of untreated or irradiated T cells were added. The cell mixtures were cultured for 7 days in the presence of PWM (1 ml cultures in duplicate) and the culture supernatants assayed for IgM RF. Neither B cells nor T cells (untreated or irradiated) synthesized detectable IgM RF when cultured alone.

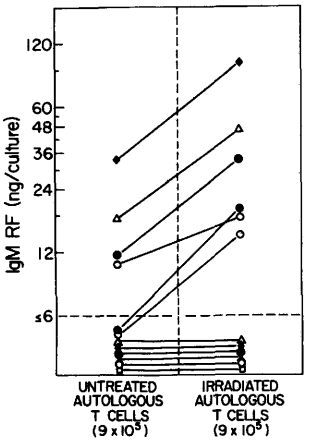

FIG. 3. Effect of irradiated versus untreated T cells on IgM RF production by autologous B cells. MNL from 12 randomly selected healthy adult donors were separated into T- and enriched B-cell fractions. B cells were cultured at 3×10^5 cells/ml in the presence of 9×10^5/ml T cells (untreated or irradiated (3000 R)) and cultured in the presence of PWM for 7 days (1-ml cultures in duplicate). Culture supernatants were assayed for IgM RF. Neither B cells nor T cells (untreated or irradiated) synthesized detectable IgM RF when cultured alone.

were consistently observed with irradiated T cells in contrast to the decline with untreated T cells (Figs. 2A and B). These results, suggesting variable degrees of suppressor T-cell influence on RF production, prompted a comparison of the effect of irradiated versus untreated T cells on RF production by autologous B cells from randomly selected healthy donors.

Effect of irradiated versus untreated T cells on RF production by autologous B cells. MNL from 12 randomly chosen healthy adult donors were separated into T- and enriched B-cell fractions. Untreated or irradiated T cells were added to autologous B cells at a ratio of 3:1 and culture supernatants assayed for IgM and IgM RF at the termination of culture. RF was not detected in supernatants from B- or T-cell fractions when cultured alone. As shown in Fig. 3, RF was detected in 4 of 12 individuals when untreated T cells were cultured with autologous B cells in the presence of PWM (9.9 ± 7.9 ng/culture). In contrast, RF production by autologous B cells could be demonstrated in 6 of 12 individuals in the presence of irradiated T cells (21.6 ± 25.8 ng/culture; $P < 0.05$). B cells from 2 of these 6 individuals elaborated RF only when cultured with autologous irradiated T cells whereas in the other 4 individuals, increases in RF production were observed with irradiated T cells in comparison to untreated T cells (Fig. 3).

No consistent pattern of change in the ratio of IgM RF to total IgM synthesis by B cells was observed with irradiated T cells versus untreated T cells (Fig. 4). In 4 of 6 cases, however, increases in the ratio of IgM RF/IgM synthesis by B cells occurred with irradiated T cells when compared to untreated T cells, suggesting disproportionate suppression of RF synthesis by untreated T cells.

Discussion. Peripheral blood MNL from a significant fraction of healthy adults contain B cells capable of synthesizing IgM RF *in vitro* (Fig. 1) (6–8, 16). Infrequent expression of RF in sera of healthy individuals argues for the presence of regulatory mechanisms governing activation of these cells. In this report, evidence is presented which indicates that radiosensitive T cells capable of suppressing PWM induced *in vitro* IgM

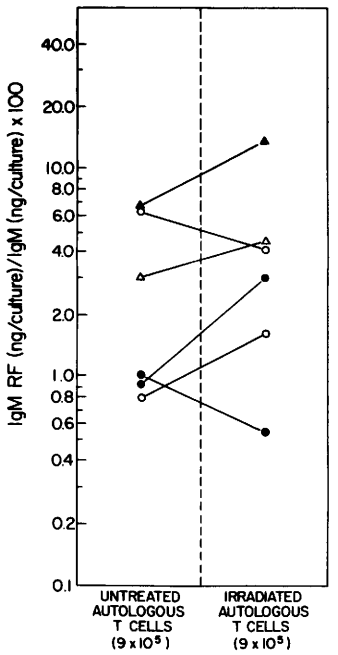

FIG. 4. Effect of irradiated versus untreated T cells on the ratio of IgM RF/IgM synthesis by autologous B cells. MNL from 12 randomly selected healthy adults were separated into T- and enriched B-cell fractions. B cells were cultured at 3×10^5 cells/ml in the presence of 9×10^5/ml T cells (untreated or irradiated (3000 R)) and cultured in the presence of PWM for 7 days (1-ml cultures in duplicate). Culture supernatants were assayed for IgM and IgM RF and the results are expressed as the ratio (IgM RF (ng/culture)/IgM (ng/culture) × 100). Only results are depicted from the 6 individuals whose B cells synthesized detectable IgM RF.

RF synthesis occur in a significant fraction of healthy adults. This conclusion is supported by dose–response analysis of irradiated versus untreated T cells in the presence of autologous B cells (Figs. 2A and B) in which consistent enhancement of IgM RF production by irradiated T cells was observed at higher T:B cell ratios. Furthermore, in 6 of 12 healthy individuals studied, enhanced IgM RF production occurred when B cells were cultured with irradiated T cells (Fig. 3). Of particular interest is the observation that B cells from 2 individuals only synthesized detectable RF in the presence of irradiated T cells. Increases in IgM RF/IgM ratios in 4/6 individuals with irradiated T cells (Fig. 4) suggests disproportionate suppression of IgM RF synthesis in these individuals and argues against "nonspecific" suppression of IgM.

Progress in the development of monoclonal antibodies directed against human T-cell antigens analogous to Lyt antigens in the mouse should soon permit identification of the T-cell subset(s) responsible for suppression of RF synthesis in these studies. In this regard, Thomas *et al.* (17) have recently demonstrated at least two distinct radiosensitive populations of T cells (an $OKT8^+$ cell and an $OKT4^+$ cell) which cooperate in suppressing PWM-induced immunoglobulin secreting cells.

The results of this study support the hypothesis that suppressor mechanisms at least partially account for *in vivo* nonexpression of RF by healthy adults. Inability to demonstrate *in vitro* RF production in 6 or 12 individuals in the presence of irradiated T cells, however, also suggests additional mechanisms are involved in the regulation of RF synthesis. Whether defects in suppressor activity for RF contribute to the enhanced RF synthesis observed in RA (7, 8) will require additional study. Analysis of regulatory mechanisms governing expression of potentially harmful immunoglobulins such as RF may eventually contribute to our understanding of "triggering" mechanisms responsible for induction of these antibodies in disease.

The author gratefully acknowledges the excellent technical assistance of Ms. Karen Seay and Maria Frangakis. The author also wishes to thank Ms. Martha Bidez for assistance in preparing this manuscript.

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Received April 21, 1981. P.S.E.B.M. 1981, Vol. 168.