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Research Article

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Antibody Synthesis by Bone Marrow Cells In Vitro following Primary and Booster Tetanus Toxoid Immunization in Humans

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Abstract. Normal volunteers received either initial or booster immunization with tetanus toxoid. Bone marrow and peripheral blood mononuclear cells were obtained for up to 28 d after immunization and were analyzed for synthesis of total Ig and specific antibodies to tetanus toxoid. Cells were cultured in vitro for 3 or 7 d with or without pokeweed mitogen (PWM). Synthesis of IgG and IgM antibodies to tetanus (IgG-Tet and IgM-Tet) and total IgG and IgM was determined by radioimmunoassay. Four functional B cell subpopulations were detected in the bone marrow after booster tetanus immunization: (*a*) B cells that spontaneously synthesized IgG-Tet appeared on day 7 after immunization but were undetectable by day 21; (*b*) B cells that synthesized IgG-Tet after stimulation with PWM appeared after day 21 and persisted for greater than 1 mo; (*c*) B cells that synthesized IgM-Tet in the presence of PWM were detectable before and after immunization; and (*d*) B cells that spontaneously synthesized IgM-Tet appeared on day 7 and were undetectable by day 21. In contrast to the other three types of bone marrow B cells described, this fourth subpopulation of PWM-independent IgM-Tet-synthesizing B cells was not detected in the peripheral blood. After primary immunization, no spontaneous antibody-producing cells were detected in the blood or bone marrow, although there was a small rise in IgM-Tet in two of three subjects. In the bone marrow, only IgM-Tet PWM-inducible cells were seen, although mitogen-responsive IgM and IgG-Tet cells were detected in the circulation. The IgM-Tet PWM-reactive cells were present even before primary antigen exposure and appear to represent the initial B cells involved in the antibody response. These data indicate that there are specific times after immunization when different functional classes of anti-Tet-synthesizing B cells and memory B cells appear in human bone marrow. Knowledge of these data may be important in developing a strategy for the transfer of immune memory from donors to recipients in the setting of bone marrow transplantation.

Introduction

The complex mechanisms whereby human B cells are induced to synthesize specific antibodies have been intensely investigated over the past decade. Recently, techniques have been developed to study in vitro synthesis of Ig and specific antibody by human B lymphocytes (B cells). Activation of human peripheral blood-derived B cells with polyclonal mitogens such as pokeweed mitogen (PWM)[1] results in T cell-dependent synthesis and secretion of Ig by B cells (1–3). Both T helper and T suppressor cells regulate this B cell response (1, 3). In humans, in vivo immunization results in the appearance of several types of functional subpopulations of B cells in the peripheral blood. Lymphoblastoid B cells are detected in peripheral blood 5–8 d after booster immunizations with tetanus toxoid (TT) antigen. These cells spontaneously synthesize IgG anti-tetanus toxoid antibody (IgG-Tet) without a requirement for T helper cells or PWM (4). Such cells are no longer detectable in the circulation by 12–14 d after immunization but are replaced by a subset of B cells that synthesize IgG-Tet in the presence of T helper cells and

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1. *Abbreviations used in this paper:* BMMC, bone marrow mononuclear cells; PBMC, peripheral blood mononuclear cells; PWM, pokeweed mitogen; Tet, anti-tetanus toxoid antibody; TT, tetanus toxoid.

PWM. The latter cells persist for up to 10 wk after immunization (4, 5).

In contrast to the IgG-Tet response, the in vitro synthesis of IgM antibody to tetanus toxoid antigen (IgM-Tet) can be induced by PWM in vitro and is not affected by in vivo booster immunization. The IgM-Tet response, like the IgG-Tet PWM response, requires T cell help (6). However, unlike the IgG-Tet response, spontaneous synthesis of IgM-Tet by peripheral blood B cells is undetectable after booster immunization (7).

In contrast to these considerable data regarding antibody synthesis by peripheral blood-derived B cells, there are few reports of specific antibody synthesis in vitro by bone marrow-derived B cells. We studied Ig and antibody synthesis and secretion by human bone marrow as well as peripheral blood mononuclear cells after primary and booster immunization of normal volunteers with TT antigen. After booster immunization, four functional B cell subpopulations in bone marrow were defined with respect to time of appearance and requirement for PWM. However, only one of these appeared in the bone marrow after primary immunization. Our data indicate that bone marrow B cells differ from peripheral B cells in regard to both IgG-Tet and IgM-Tet synthesis during both a primary and secondary specific antibody response.

Methods

Immunization. Five normal Oriental volunteers, 25–30 yr of age, who had never received TT immunization, served as the subjects for primary immunization. Subjects for the reimmunization (booster) studies were four normal volunteers, 30–40 yr of age, who had received routine TT immunization during childhood. Each subject received a single intramuscular booster immunization of 0.5 ml (5 flocculating units) of soluble TT (Lederle Laboratories, Pearl River, NY). Peripheral blood and bone marrow samples were obtained before and at intervals of 7–28 d after immunization.

Bone marrow cells and blood lymphocytes. Bone marrow cells were obtained from the posterior iliac crest by needle aspiration. Bone marrow (4–6 ml) was drawn in a plastic syringe containing 1,000 U of preservative-free heparin. Bone marrow mononuclear cells (BMMC) were prepared by Ficoll-Hypaque density-gradient centrifugation. Peripheral blood mononuclear cells (PBMC) were isolated by the same technique and washed three times with RPMI 1640 before use.

Cell cultures. Optional number BMMC (1,000,000) or PBMC (2,000,000) were cultured in RPMI 1640 medium supplemented with glutamine (6 mM), gentamicin (0.04 mg/ml), and 15% heat-inactivated fetal calf serum. Cultures containing a volume of 1.5 ml were performed in 16 × 125-mm plastic tubes (Falcon 3033, Falcon Labware, Oxnard, CA) with or without PWM (Gibco Laboratories, Grand Island, NY) at a final dilution of 1:100 (vol/vol) and with or without cycloheximide (50 μg/ml) (Sigma Chemical Co., St. Louis, MO). Tubes were incubated in a humidified atmosphere at 37°C with 5% CO_2 and harvested on day 3 or 7. In some experiments, B cells (400,000) were cultured with irradiated (1,500 R) T cells (1,600,000). T and B cells were separated by differential sedimentation of T cells rosetted with 2-amino-ethyl-isothiouronium bromide treated sheep erythrocytes as previously reported by us (1). PMMC B cells obtained in this fashion are devoid of T cells to the degree that alone they will not under go PWM-induced Ig synthesis.

Radioimmunoassay. Quantitative radioimmunoassays for total IgG and IgM and IgG-Tet and IgM-Tet synthesized in vitro were performed in microtiter plates (Scientific Products Inc., Baltimore, MD) as previously described (7).

The radioimmunoassays for IgG-Tet and IgM-Tet were identical to the total IgG or IgM radioimmunoassay except that purified TT (a kind gift from Wyeth Laboratories, Marietta, PA) at a concentration of 1 mg/ml was used for the initial coating of the microtiter plates (7).

For each radioimmunoassay a seven-point standard curve of IgG or IgM ranging from 2.5 to 50 ng was performed in parallel with the culture samples on each plate and the value for IgG or IgM extrapolated from the standard curve. Antibody to TT was also determined by comparison to the standard curve. The lower limit of detection for anti-TT antibody produced in vitro was 1 ng/culture. Standardization of specific antibody concentration may be underestimated by this procedure (6) although all batches of antisera did not show this effect when assayed against known amounts of TT antibody. This may account for some (but not all) of the discrepancy seen in specific vs. total Ig seen with spontaneous synthesis. PWM-induced synthesis of Ig or antibody to TT was determined by subtracting the quantities synthesized in cultures of cells alone from the amount produced in cultures containing PWM. The data are expressed as nanogram per 1.5 ml of culture and microgram per milliliter of serum. Individual values given in tables and figures represent the mean of duplicate cultures. The variability between cultures was generally <10%.

To check the specificity of the IgM-Tet of samples obtained from individuals before primary immunization, 25 μg of TT was added to each radioimmunoassay well in duplicate wells with the samples so as to block all specific binding. The results for these experiments are expressed as percent inhibition.

Results

Serum antibody responses. IgG-Tet was not detectable before or after a single primary TT immunization. IgM-Tet was detected (7.4 and 31.0 μg/ml maximum) between days 7 and 28 in two of three subjects assayed. The serum antibody response after booster immunization has been well studied with peak IgG-Tet responses (up to 250 μg/ml) between 10 and 21 d and no change in the low levels (~5 μg/ml) of IgM-Tet.

Primary immunization

Spontaneous synthesis of IgM-Tet, IgG-Tet, and Total Ig. After primary immunization, there was no detectable spontaneous synthesis of IgG-Tet or IgM-Tet by BMMC or peripheral blood B cells from days 0 through 28. It is possible that a response occurred between the weekly time points, although there was no suggestion of spontaneous production in any subject. The levels of total IgG produced did not differ significantly from those of reimmunization subjects (see below).

PWM-induced synthesis of IgM-Tet. In contrast to results of spontaneous IgM-Tet and IgG-Tet, PWM-induced IgM-Tet synthesis was detectable in the primary response. Indeed, both PBMC and BMMC B cells, in the presence of PWM and irradiated T cells, synthesized small quantities of IgM-Tet before immunization (Table I). It is important to emphasize that the history of not receiving TT immunization was carefully estab-

Table I. PWM-induced In Vitro Synthesis of IgG-TET and IgM-TET (Nanogram per Culture) following Primary Immunization

	Days postimmunization							
	PBMC* (IgG-Tet/IgM-Tet)				BMMC (IgG-Tet/IgM-Tet)			
Subject	0	7	21	28	0	7	21	28
H	0/18	0/45	0/10	3/21	0/9	0/56	2/31	0/2
K	0/14	0/21	ND	4/13	0/5	0/18	ND	0/5
M	0/21	4/32	ND	3/19	ND	ND	ND	ND
R	ND	0/16	ND	15/23	ND	0/7	ND	0/16
T	0/15	ND	ND	ND	ND	ND	ND	ND

ND, not determined.
* Cultures consisted of 400,000 non-T cells plus 1,600,000 irradiated T cells.

lished in these Japanese individuals. Primary immunization did appear to enhance this PWM-induced IgM-Tet synthesis (17±1.3 ng/culture on day 0, 28±4.7 ng/culture on day 7, and 19±1.8 ng/culture on day 28 for PBMC) although it did not reach statistical significance because of the small number of subjects. We sought to assess whether the IgM-Tet we detected was truly anti-Tet or if it was an artifact as it was present before known immunization. Culture supernatants from two subjects' PBMC and BMMC were retested by radioimmunoassay with a duplicate assay done in the presence of 25 μg/well (125 μg/ml) of soluble TT. This amount of TT is sufficient to block any TT antibody from binding in our assay and thus inhibition could be taken as a measure of TT-specific antibody. Nonspecific inhibition was corrected for by blocking with bovine serum albumin. TT blocked the IgM-Tet detected from BMMC by 70% and from PBMC by 57%. Furthermore, cycloheximide controls (75 μg/ml) were included in all cultures to exclude carried over antibody.

PWM-induced synthesis of IgG-Tet. Peripheral blood B cells from nonimmunized individuals, when cultured with PWM plus irradiated T cells, failed to produce IgG-Tet before primary immunization. However, at day 28, all subjects' B cells, in the presence of PWM and T cells, produced small amounts of IgG-Tet (6.3 ng±3.0) (Table I). BMMC from the same subjects (days 0–28) failed to produce IgG-Tet under similar conditions although they synthesized normal amounts of total IgG (e.g., 1,965±479 ng/culture at day 28).

Booster immunization

Spontaneous synthesis of IgG-Tet and total IgG. The time course of in vitro IgG-Tet and total IgG synthesis by BMMC after booster immunization is shown in Fig. 1 *A* and *B*. BMMC from normals receiving no booster immunization synthesized no or minimal IgG-Tet when cultured in vitro for 7 d (0–8 ng). Synthesis of IgG-Tet was substantially increased to 237–953 ng 1 wk after TT immunization and returned to base-line levels by 21 d. Synthesis of total IgG (including IgG-Tet) was 827–2695 ng in normal controls at day 0. This increased to 1.6–2.6-fold 7 d after TT immunization, returning to base line by day 21. Total IgG synthesis after booster immunization increased more than IgG-Tet (mean: 2,000 ng vs. 656 ng); at the peak of the spontaneous IgG-Tet response on day 7, IgG-Tet synthesis accounted for 16–44% of total IgG synthesis.

1 wk after booster immunization, BMMC from normal individuals synthesized 65–86% of the IgG-Tet within the first 3 d in culture, while 14–54% of the total IgG appeared in this period. Thus, in 3 d, 39–41% of the total IgG was accounted for by IgG-Tet. The in vitro increase in spontaneous non-TT-specific IgG and IgM seen after booster immunization thus occurred primarily later than specific antibody synthesis. While the reason for this increase in total Ig is unknown, it may be due to nonspecific helper effects derived in vivo from the activated tetanus helper cells as shown by Welch et al. (8).

We directly compared spontaneous IgG-Tet synthesis by BMMC and PBMC from the same donors 7 d after booster immunization (Table II). The percent of specific antibody (IgG-Tet to total IgG) was lower for BMMC than for PBMC (mean: 25 vs. 54%). Synthesis of both total IgG and IgG-Tet were inhibited >95% by cycloheximide, which indicates that antibody synthesis occurred in vitro and was not carried over on cell surfaces or as preformed cytoplasmic antibody (data not shown).

PWM-induced synthesis of IgG-Tet and IgG. After booster immunization, BMMC stimulated with PWM failed to synthesize detectable (i.e., greater than spontaneous synthesis alone) IgG-Tet until 21 d after immunization (Fig. 2 *A*). PWM-induced IgG-Tet synthesis increased to 116–648 ng by day 28 postim-

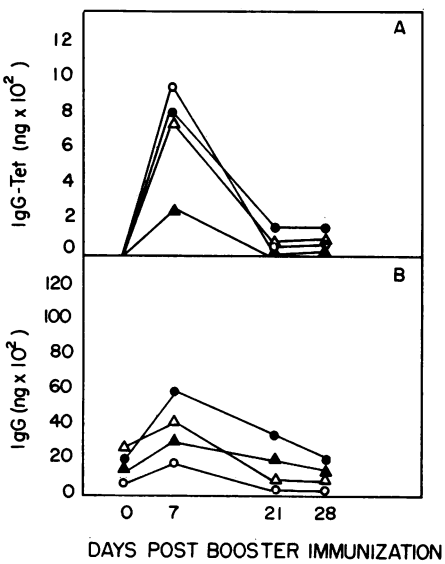

Figure 1. Spontaneous synthesis of IgG-Tet and total IgG by BMMC. 1,000,000 cells from four normal individuals obtained 0–28 d after in vivo booster immunization were cultured for 7 d and both IgG-Tet (*A*), and total IgG (*B*) synthesis were determined by radioimmunoassay. Different symbols represent different individuals tested.

Table II. Comparison of IgG-Tet and Total IgG Synthesis in Bone Marrow and PBMC after Booster Immunization

	IgG-Tet (*ng*)				Total IgG (*ng*)				IgG-Tet/Total IgG (%)			
Cells*	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean
Bone marrow	705	780	953	813	4,502	5,645	2,157	4,101	10	14	44	25
Peripheral blood	1,045	1,100	1,695	1,297	2,183	4,230	1,974	2,796	50	26	86	54

* BMMC (1,000,000) or PBMC (2,000,000) from the same individuals obtained 7 d after in vivo booster immunization were cultured for 7 d without PWM and both IgG-Tet and Total IgG synthesis were determined by radioimmunoassay.

munization. PWM-induced total IgG synthesis ranged between 893 and 9,373 ng before immunization; there was no detectable change in total IgG synthesis over this period (Fig. 2 *B*). The percent of PWM-induced IgG accounted for by IgG-Tet was consistently <9% for up to 28 d after immunization.

Spontaneous synthesis of IgM-Tet and IgM. Before booster immunization, BMMC showed no spontaneous synthesis of IgM-Tet when cultured in vitro. 1 wk after immunization, BMMC showed a substantial increase of 10.7–28.8 ng of IgM-Tet (Fig. 3 *A*). Spontaneous synthesis of IgM-Tet was minimal or absent on day 21, as occurred with spontaneous synthesis of IgG-Tet. PBMC from these same individuals failed to make detectable IgM-Tet at the same times (results not shown). Total IgM synthesis by BMMC alone ranged between 103 and 354 ng before immunization and appeared to change in parallel to spontaneous synthesis of IgM-Tet (Fig. 3 *B*). The percent of total IgM accounted for by IgM-Tet ranged between 2.1 and 3.4% at the peak of the spontaneous IgM-Tet response (day 7).

PWM-induced synthesis of IgM-Tet and IgM. Just before booster immunization, BMMC synthesized 2.7–17.4 ng of IgM-Tet in vitro in the presence of PWM (Fig. 4 *A*). Although only two of the three individuals' BMMC cultures with PWM made readily detectable quantities of IgM-Tet, all three IgM-Tet culture levels were clearly above background (Fig. 3 *A*). This PWM-induced IgM-Tet synthesis increased slightly 3 or 4 wk after immunization. Total IgM synthesis by BMMC with PWM was unaffected by booster immunization (Fig. 4 *B*). The percent of PWM-induced IgM accounted for by PWM-induced IgM-Tet was consistently <6% and unaffected by booster immunization.

Effect of a second immunization after primary antigen exposure. We studied PBMC from one subject who received a

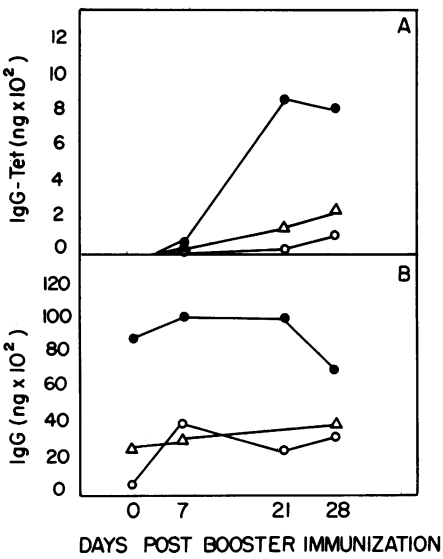

Figure 2. PWM-induced synthesis of IgG-Tet and total IgG by BMMC. 1,000,000 cells from three normal individuals obtained 0–28 d after in vivo booster immunization were cultured for 7 d with PWM and both IgG-Tet (*A*) and total IgG (*B*) synthesis were determined by radioimmunoassay. PWM-induced synthesis was determined by subtracting the quantities synthesized in cultures of cells without PWM. Different symbols represent different individuals tested.

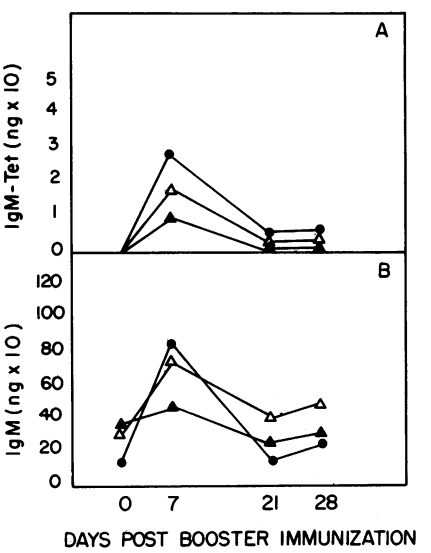

Figure 3. Spontaneous synthesis of IgM-Tet and total IgM by BMMC. 1,000,000 cells from three normal individuals obtained 0–28 d after in vivo booster immunization were cultured for 7 d and both IgM-Tet (*A*) and total IgM (*B*) synthesis were determined by radioimmunoassay. Different symbols represent different individuals tested.

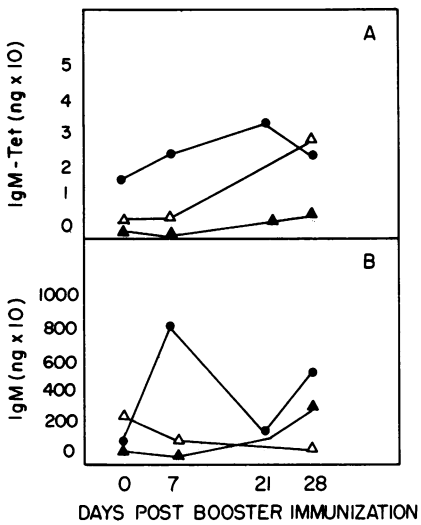

Figure 4. PWM-induced synthesis of IgM-Tet and total IgM by BMMC. 1,000,000 cells from three normal individuals obtained 0–28 d after in vivo booster immunization were cultured for 7 d with PWM and both IgM-Tet (*A*) and total IgM (*B*) synthesis were determined by radioimmunoassay. PWM-induced synthesis was determined by subtracting the quantities synthesized in cultures of cells without PWM. Different symbols represent different individuals tested.

second TT injection 5 mo after his primary TT injection. However, as TT is typically given as a series of three immunizations for developing initial immunity, this second injection was not meant to be equivalent to a booster immunization. Serum IgM-Tet was present on the day of the second challenge (0.9 μg/ml) and showed no change over the subsequent 80 d (range 0.7–1.8 μg/ml). Serum IgG-Tet was not detectable at day 0 but became so at day 21 (10 μg/ml), rose to 23 μg/ml by day 36, and was still present at day 80 (6 μg/ml). B cells spontaneously secreting IgM or IgG-Tet were not detected in blood at days 7, 21, or 28 after the second immunization. In contrast, PWM-inducible B cell production of IgG-Tet rose from very low levels at day 0 (2.8 ng) to 9.8 ng by day 21. This is still quite low compared with the levels seen after booster reimmunization. IgM-Tet that was PWM stimulated was produced at day 0 and showed no significant change after the second injection (mean 28.2 ng). These levels of PWM-induced IgM-Tet are higher than seen with this individual after the primary immunization (mean 12.7 ng) and similar to that seen in the booster-immunized subjects.

Discussion

Despite the central role of the bone marrow in evolution of the B lymphocytes axis and development of antibody responses, there have been relatively few studies of specific antibody synthesis by human bone marrow cells (9, 10). Reports on primary immunization have been with blood cells after in vitro immunization (11–14). Data on human bone marrow synthesis of antibody after initial antigen exposure have not been reported. BMMC from patients with rheumatoid arthritis have been reported to synthesize rheumatoid factor (15), while BMMC from patients with chronic immune thrombocytopenic purpura have been reported to synthesize autoantibodies to platelets (16). There are, however, no reports of specific antibody synthesis by human bone marrow cells in relationship to defined in vivo immunization.

We immunized four, normal, previously nonimmune and four immune volunteers with a standard immunization of TT and studied in vitro synthesis of total Ig and specific antibodies to TT by bone marrow and PBMC. After the booster immunization, we found four functional B cell subpopulations in bone marrow with respect to time of appearance and requirement for PWM: (*a*) IgG-Tet "spontaneously" synthesizing cells that are present on day 7 do not require PWM and are undetectable by day 21; (*b*) IgG-Tet-synthesizing cells that require PWM and T cells and appear by day 21; (*c*) IgM-Tet-synthesizing cells that are detectable before and after booster immunization and required PWM and T cells; and (*d*) IgM-Tet-synthesizing cells that appear on day 7 persist for up to 21 d and are PWM independent. These subsets are probably maturational stages in the antibody response and should not be regarded as those in mutually exclusive pathways. After primary immunization, a small PWM-inducible IgG-Tet response was found in the blood at day 21, while IgM mitogen-reactive B cells could be detected in BMMC (and blood) before and after initial antigen exposure. The appearance of these various functional B cells is summarized in Table III.

Our blocking experiments suggest the IgM-Tet detected before antigen exposure was true antibody. It might result from either the earlier expansion of low affinity TT-reactive cells by

Table III. Summary of Functional B Cell Responses after Primary and Booster Immunization

	Serum		Spontaneous		PWM inducable	
	IgM	IgG	IgM	IgG	IgM	IgG
	Primary response					
Blood	+	−	−	−	+	+ (little and late)
Bone marrow			−	−	+	−
	Booster response					
Blood	−	+	−	+	+	+
Bone marrow			+	+	+	+

cross-reacting antigens or from the ability of PWM to induce "primary" B cells for IgM production. The latter explanation is supported by the fact that PWM has been reported to induce in vitro an IgM anti-sheep erythrocyte antibody response before antigen exposure (14). Also although antibody to keyhole limpet hemocyanin has not been reported in culture of human lymphocytes stimulated with PWM, low levels of anti-keyhole limpet hemocyanin antibody have been detected in serum before immunization (17). The absence of spontaneous antibody-producing cells in the bone marrow after initial antigen exposure is consistant with the hypothesis that such cells represent the progeny of recently activated memory cells (18). This type of B cell would not be represented in the primary humoral response nor reflect B cells initially stimulated by antigen.

One concern in analyzing our data relates to the level of contamination of bone marrow samples with peripheral blood-derived B cells. In previous studies, we have investigated this problem in detail (19). For example, using ^{51}Cr-radiolabeled erythrocytes we found a maximum contamination of similarly obtained bone marrow samples with peripheral blood T cells of <20%. B cell contamination would be comparatively less, probably <5%. Others have reported similar data (20). In the present study, we detected spontaneous IgM anti-Tet antibody synthesis in bone marrow but not peripheral blood; a finding incomparable with the notion of the results being due to peripheral blood contamination. Furthermore, while investigating primary responses in blood and bone marrow we saw a late IgG anti-Tet PWM response phase not present concurrently in the bone marrow. These findings argue strongly against the concept that events detected in the bone marrow merely reflect blood cell contamination of the samples.

The functional response of bone marrow B cell compartment to in vivo TT immunization was distinct from that of peripheral blood B cells. IgG-Tet PWM-responsive B cells appeared in the circulation 28 d after primary immunization but were not detected in the bone marrow. After booster reimmunization, spontaneous synthesis of IgM-Tet that was readily detectable in bone marrow cells was not present in peripheral blood cells (7). This most likely represents the fact that only certain differentiation stages of B cells can be found circulating, while a different array of maturational B cells develop in the bone marrow. This latter effect may reflect the role of bone marrow in maintaining ongoing antibody synthesis to a variety of antigens.

Studies of early splenectomy in mice indicated that the migration of plaque-forming cells from the spleen to the bone marrow occurs within 2 d after booster immunization with sheep erythrocytes (21). In guinea pigs, influx of lymphoblastoid cells into the bone marrow via the blood stream occurs within 3 d after a single dose of intravascular antigen administration (22). Our finding of PWM-inducible IgG-Tet-producing cells in the blood after primary immunization without similar cells in the bone marrow suggests that the initial differentiation of cells for the IgG-Tet response after peripheral inoculation occurs outside of the bone marrow. Furthermore, lymphoblastoid cells that synthesize IgG-Tet spontaneously are also detected in human peripheral blood after TT booster immunization (4). It is likely that the influx of lymphoblastoid cells derived from lymph nodes and/or spleen into bone marrow occurs via the blood in humans and these cells are responsible for subsequent antibody synthesis detected in the bone marrow. These lymphoblastoid cells disappear from the peripheral blood by day 14 (4) and from the bone marrow by day 21 after booster immunization. Since considerable levels of IgG-Tet are detectable in the serum for years after immunization (23) this ongoing synthesis of IgG-Tet may occur in tissues other than the bone marrow such as lymph nodes and spleen. Alternatively, long-term anti-TT production may result from active bone marrow B cells that have such a low frequency that we have failed to detect them due to sampling error. The percentage of spontaneous specific anti-TT antibody produced by BMMC was 10% for IgG and 3% for IgM and likely reflects the fact that BMMC are actively involved in synthesis of many antibodies at any one time.

Spontaneous synthesis of IgM-Tet is not detected in human peripheral blood after booster immunization. In contrast, it is clearly present in bone marrow cells. One possible explanation for this disparity is that there are so few spontaneous IgM-Tet-synthesizing cells in peripheral blood that they cannot be detected during in vitro culture. Alternatively, IgM-Tet-synthesizing lymphoblastoid cells could arise in the bone marrow without a prior blood-borne phase as the bone marrow provides the major environment for B cell development. The bone marrow response to booster immunization by cells making IgM-Tet also differs from the response of cells synthesizing IgG-Tet as reflected in serum antibody levels where there is a substantial increase in IgG-Tet but not IgM-Tet after booster immunization. The most likely explanation of this disparity is that the spontaneous IgM-Tet-producing cells do not synthesize sufficient quantities of IgM-Tet to cause a detectable increase in the serum level of IgM-Tet but may provide a pool of progenitor cells that give rise to IgG antibody-synthesizing cells analogous to the PWM-responsive IgM-Tet B-cells. It is possible that between day 7 and 28 we missed a small rise and fall due to the rapid half life of IgM.

Our data suggest that coincident with intramuscular primary antigen exposure that was sufficient only to increase IgM-Tet levels, there was stimulation of PWM-responsive IgM-Tet cells, cells already present before known antigen exposure. Toward day 28, PWM IgG cells appear in the circulation but not the marrow, which reflects IgG memory cells produced in the lymphoid tissues. When activated by a second immunization, they gave rise to a small IgG serum response. These events appear to be quite distinctive from events occuring with a booster reimmunization where spontaneous antibody-producing cells were found 7 d later in both the blood (IgG) and the bone marrow (IgG and IgM). These IgG-Tet cells likely represent the cells actively disseminating and producing the early (day 7) and vigorous IgG-Tet response. Of interest is the fact that quantitatively the BMMC produced up to 10-fold more IgG-Tet on a per B

cell basis as compared with PBMC. As no rise in serum IgM-Tet was seen, the spontaneous IgM-Tet cells in the marrow probably served as a precursor pool (either by isotype switch or by further division and differentiation. Again the IgG-Tet responsive cells seen afterward were a reflection of an activated short-term memory.

Studies in rodents suggest that under some circumstances it is possible to transfer immune memory from donor to recipient by the transplantation of immune spleen cells (24). In other circumstances, immune memory is lost or impaired after adoptive transfer. In humans undergoing bone marrow transplantation it has been difficult to transfer immune memory to antigens such as purified protein derivative or Candida (25, 26). Recipients of allogeneic HLA-A, B, C, and D-identical bone marrow transplants or transplants from genetical identical twins show profound immune deficiency for 6–12 mo after transplantation (10, 27–29). Most data indicate that these patients respond abnormally to antigens and immunization with various antigens (29, 30) and in a fashion similar to that seen with primary TT immunization (unpublished data). One approach to this problem would be to immunize the donor to specific antigens at an appropriate time before transplantation. The data presented herein indicate that there are specific times after booster immunization when putative antibody-synthesizing cells and memory cells can be found in the bone marrow.

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