INDUCTION OF IMMUNOLOGIC TOLERANCE IN OLDER NEW ZEALAND MICE REPOPULATED WITH YOUNG SPLEEN, BONE MARROW, OR THYMUS

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We previously reported that 6-8-wk old NZB and NZB/NZW F1 (B/W) hybrid mice are relatively resistant to tolerance induction by both ultracentrifuged bovine (BGG) and human gamma globulin (HGG) (1). We also noted that immunized NZB and B/W mice made a higher titered antibody response than did NZW, Balb/c, C3H, and C57Bl mice. Similarly, Weir et al. immunized NZB mice with soluble bovine serum albumin and found an enhanced antibody response and some difficulty in tolerance induction, compared to control CBA and C57Bl X CBA F1 mice (2). In a preliminary report, we showed that 15-17-day old NZB and B/W mice, in contrast to the 6-8-wk old animals, could initially be rendered unresponsive to BGG but that they rapidly lost this tolerance (3). A series of experiments was therefore devised to further investigate and extend this latter observation. In this paper, we report the successful induction of tolerance in older thymectomized and irradiated mice repopulated with lymphoid cells from young animals.

Materials and Methods

Animals.—Mice of both sexes, aged 2½–6 wk, were obtained from inbred stock colonies maintained at the National Institutes of Health (NIH). Strains used in these experiments were NZB, B/W, C3H, C57Bl, and Balb/c.

6-wk old B/W F1 and Balb/c mice were used in the biofilter experiments. Cell transfer studies involved only B/W animals. Recipient B/W mice were 2-3 months of age. Old donor mice were 3-4 months of age, while young donor mice were 12-15 days of age. All animals were housed in NIH animal facilities and those which were irradiated received a 10 day course of oxytetracycline in their drinking water (0.5 g/liter).

Antigens.—Pretreatment antigens were bovine gamma globulin Cohn fraction II (Mann Research Laboratories, Inc., New York) and egg albumin (Ea) crystallized twice (Worthington Biochemical Corp., Freehold, N. J.). Antibody assays were performed using chromatographically pure 7S bovine gamma globulin (Mann). This material at a concentration of 10 mg/ml gives only a single precipitin line after immunoelectrophoresis with rabbit anti whole bovine serum (4).

Tolerance Induction.—Mice were pretreated with varying doses of ultracentrifuged BGG or Ea. Proteins were prepared in a Spinco 40 rotor at 105,000 g for 30 min as previously described (1).
Single injections: Newborn mice less than 48 hr old received 8.0 mg of soluble BGG given into the dorsal subcutaneous tissues in a 0.25 cc volume of normal saline through a 30 gauge needle. Young mice 7–9 or 16–18 days of age received 1.6 mg soluble BGG/g of body weight, given intraperitoneally. In one experiment with mice aged under 3 wk or over 6 wk, animals were treated with 10.5 or 24.5 mg of ultracentrifuged BGG. Soluble Ea, 8.0–10.0 mg, was given to littermates in all experiments as a specificity control.

Multiple injections: 3 to 6 day old B/W and C3H mice were given biweekly injections of 10–15 mg of ultracentrifuged BGG or 5 mg of EA intraperitoneally for 5 wk, then challenged 12 or 28 days later with BGG in Freund's adjuvant.

In utero injection: Full-term pregnant NZB females carrying B/W hybrid litters were given a single injection of 60 mg of soluble BGG intraperitoneally. Offspring were challenged at 1 month of age and bled 1 and 3 months later.

Challenge.—Young animals were challenged on the 28th day of life. Older animals receiving single injections of BGG were challenged 12 days after pretreatment. Mice treated with multiple injections were challenged either 12 or 28 days after the last dose of soluble BGG was given. The challenge regimen was 1.0 mg of BGG in complete Freund's adjuvant (Difco Laboratories, Detroit, Mich.) equally distributed over four footpads in a total volume of 0.2 cc.

Antibody Assay.—Mice were regularly bled at 2 wk intervals postchallenge. Antibody titers were assayed by standard microtiter hemagglutination methods using tanned formalized sheep cells (4, 5). All sera from a single experiment were studied on the same day. Known positive and negative sera were included with each assay.

Biofiltration.—Biofiltration experiments were performed after the method of Mergenhagen et al. (6). Donor B/W and Balb/c mice were given 10 mg of crude BGG or Ea intravenously and 16 hr later sacrificed by cervical dislocation. Then 0.2 cc serum containing 1–2 mg BGG was given intraperitoneally into recipient animals of both strains. These recipient mice were challenged with 2.5 mg of BGG in complete Freund's adjuvant given intraperitoneally 8 days later. They were bled at 8, 19, and 34 days postchallenge.

Serum Transfer.—2-month old NZB or C57Bl mice were used as serum donors. Animals were bled, clot retraction allowed to occur at room temperature, and serum removed and immediately frozen at −70°C overnight. The next day, 0.25 cc of serum from one or the other strain were added to either 10 mg of soluble BGG or EA in 0.25 cc saline and the combination was given intravenously to 6-wk old C3H mice. These mice were challenged 12 days later and bled at 13 and 40 days.

Thymectomy.—Thymectomy of 2–3 month old B/W mice was performed under a dissecting microscope with the animals under pentobarbital anesthesia (Diabutal). Each mouse received 0.01 cc/g of body weight of an 8 mg/cc stock pentobarbital solution intraperitoneally. Thymuses were removed through a midline sternal incision with a glass suction catheter and the wound closed with Michel wound clips. Examination of random animals (about 15%) at the termination of the studies revealed no thymic remnants.

Irradiation.—All thymectomized mice were irradiated 5–7 days post-thymectomy in lucite boxes holding 7–9 animals/box. Irradiation was performed with a double port Westinghouse 200 Kv, 15 mA machine. Half-value layer (HVL) = 0.9 mm Cu. Distance from source was 54 cm and the rate of dose delivery was 139 R/minute. All mice received 850 R over 6.11 min.

Some individual thymus glands from donor animals were irradiated with a dose of 2000 R given at 675 R/min. This dose is adequate to kill all the cells contained in the gland (7).

Cell Suspensions.—Animals were killed by cervical dislocation. Spleens were removed, trimmed, and placed into cold Eagle's medium without calcium, magnesium, or antibiotics (NIH media unit). They were next teased into small fragments in plastic Petri dishes and the dish contents were poured into 40 cc conical glass test tubes. The supernatant cells were sub-
sequently aspirated through 20 and 23 gauge needles, centrifuged at 500 g for 5 min at 10°C, and resuspended in appropriate volumes.

Bone marrow cells were obtained by flushing Eagle's medium through femurs and tibiae of donor mice; then they were handled as were spleen cells. Cell counts were performed in a Levy counting chamber using 1% acetic acid diluent.

**Thymus Grafts.**—Whole thymus grafts were placed through a small incision into a pocket in the axillary subcutaneous tissue of recipient mice. Ether anesthesia was used; a single wound clip closed the incision.

**Cell Transfer.**—Cell transfer experiments consisted of either spleen cell or whole thymus-bone marrow cell transfers. In one study, thymectomized irradiated recipients received \(18 \times 10^6\) spleen cells intravenously from either young (12-15 days) or old (2-3 months) donors. In a second study, groups of 10-12 thymectomized irradiated mice were given various combinations of either young or old whole thymus grafts or bone marrow cells (8 \( \times \) \(10^6\) cells intravenously). These combinations were (a) young thymus and young bone marrow; (b) old thymus and young bone marrow; (c) young thymus and old bone marrow; (d) old thymus and old bone marrow; (e) old irradiated (2000 R) thymus and bone marrow.

On the day after cell transfers, mice were given either 10 mg soluble BGG or 8 mg soluble Ea intraperitoneally. Challenge, bleeding schedules, and tanned cell hemagglutination assay for anti-BGG activity were then carried out as previously described (1).

**RESULTS**

**Young and Old Mice.**—Young NZB mice developed a transient state of tolerance to BGG, whereas BGG-treated older NZB and B/W mice made a brisk antibody response like that of Ea-treated littermates (Fig. 1). Young mice given Ea made as much antibody as older mice similarly treated.

**Young Escape.**—To determine whether or not escape from tolerance was unique to young NZB mice, a second experiment was performed comparing C3H, Balb/c, C57Bl, and NZB mice 15-18 days of age. All mice received either ultracentrifuged BGG (1.6 mg/g body weight) or Ea (8.0 mg) intraperitoneally and were challenged with BGG in adjuvant at 28 days of age.

Only the NZB mice lost their immunological unresponsiveness over time, showing a rapid recovery that was maximal at 56 days postchallenge (Fig. 2 and top of Table I). The C3H, Balb/c, and C57Bl mice continued to be almost totally tolerant even at 160 days of age.

The data for Ea-treated control animals from the same experiment is shown in Fig. 3. The rate of antibody formation in the NZB mice exceeded that in the other three strains, and higher titers were attained. The control strains reached peak titers at the same time as the NZB, but the levels were lower.

**Newborn Escape.**—Mice were pretreated with either BGG or Ea, 8 and 5 mg, respectively, at less than 48 hr or 7-9 days of age (Table I, bottom). Challenge was again at 28 days of life. Pretreatment of New Zealand mice with BGG at these early ages was not sufficient to maintain a long-term state of tolerance as it was in the C3H controls. By 8 wk postchallenge, mean titers in NZB and B/W mice were greater than half that of Ea-treated littermates, whereas they were 1/6 in pretreated C3H mice relative to Ea-treated C3H controls. NZB and B/W
control animals again had higher antibody titers than did C3H controls, as in Fig. 3.

Tolerance Induction In Utero.—Tolerance was induced in B/W offspring of NZB mothers who received BGG during pregnancy (Table II). Pregnant NZB females were treated with a single injection of 60 mg of soluble BGG; controls were untreated. Offspring were born 3–4 days later and challenged at 1 month of age. Those from untreated mothers made antibody, whereas those from BGG-treated mothers were tolerant. Tolerant and control mice made comparable amounts of antibody 3 months after challenge.

Tolerance Maintenance.—In an attempt to induce and then maintain tolerance, BGG was given in multiple injections (twice weekly for 5 wk) starting with mice 3–6 days old. The results after challenge at 12 or 28 days were the same and are combined in Fig. 4. In contrast to a single injection of BGG, repeated doses of BGG given from birth were successful in inducing and maintaining a state of partial tolerance to this antigen. Mean titers in B/W mice were less than one-third that of Ea-treated littermates and remained depressed throughout the 12 postchallenge wk of study. As in the single injection experiments (Figs. 1 and 2), the timing of the (abortive) escape was again 2–4 wk postchallenge. The C3H mice showed an even greater depression and made virtually no antibody.

Biofilter Studies.—Of several adult mouse strains studied, Balb/c are difficult to render tolerant to ultracentrifuged HGG (1, 8) because they are hyperphagocytic and are immunized by trace amounts of microaggregated protein. Balb/c mice are also more resistant to tolerance induction with BGG.¹ Unlike New

¹ Staples, P. J., and N. Talal. Unpublished observations.
Zealand mice, however, Balb/c can maintain tolerance when it is induced in very young mice (Fig. 2). They also develop tolerance if the aggregates are removed by biofiltration (8). A cross-strain biofiltration experiment was performed to compare NZB and Balb/c mice.

Biofiltration of BGG through Balb/c mice prior to injection into other Balb/c mice renders the latter recipient animals tolerant (Fig. 5, open squares). However, biofiltration of BGG through either Balb/c or NZB mice prior to injection into other NZB mice fails to render these latter recipient animals tolerant (Fig. 5, closed circles). The slope of antibody production in these recipient mice was the same as in Ea-treated animals (Fig. 5, open circles).

Thus, no loss of antibody response was noted when BGG was biofiltered through either NZB or Balb/c mice and then given back into other NZB animals. Since the BGG was sufficiently cleared of aggregates to induce tolerance in Balb/c mice, it seems likely that the mechanism responsible for lack of tolerance in NZB mice must be different than the hyperphagocytosis operative in the Balb/c strain.

Serum Transfer.—This experiment ruled out an NZB serum factor as responsible for the relative resistance to tolerance. 2-month old NZB and C57Bl mice were sacrificed, and 0.25 cc of fresh frozen serum from each strain was combined with an equivalent volume of either 10 mg of soluble BGG or Ea. 6-wk old C3H mice received these combinations intravenously, were subsequently challenged, and bled. Neither NZB nor C57Bl serum interfered with the induction of tolerance in the C3H mice.

Spleen Cell Transfer.—Old (2-3 month) thymectomized and irradiated (850 R) B/W recipients were repopulated with either $18 \times 10^6$ young (12-15 days) or $18 \times 10^6$ old (3-4 months) spleen cells given intravenously postirradiation.

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**Fig. 2.** Tolerance escape curves over a period of time in 3-wk old NZB and control strains which were given 8–10 mg soluble BGG at 16–18 days of age. All points represent mean titers of 6–13 mice.
TABLE I
Loss of Specific Immunological Tolerance to BGG in Neonatal and Infant NZB and B/W Mice

| Treatment Group | Mean hemagglutination titer (log.) to BGG (wk postchallenge)* |
|-----------------|-------------------------------------------------------------|
| Strain          | Age at pre-treatment | Antigen | Injected dose | No. of mice** | 2 wk | 4 wk | 6 wk | 8 wk | 10 wk |
| Young Escape:   |                 |         |              |               |      |      |      |      |       |
| NZB 17-18       | 17-18            | BGG     | 12-14        | 13            | 0.3  | 2.2  | 3.9  | —    | 5.9   |
| C3H 16-19       | 16-19            | "       | 10-12        | 10            | 0.0  | 0.4  | 0.5  | —    | 0.6   |
| Balb/c 15-18    | 15-18            | "       | 10-12        | 10            | 0.0  | 0.1  | 0.3  | —    | 0.3   |
| C57Bl 16        | 16               | "       | 9-12         | 8             | 0.0  | 0.0  | 0.0  | —    | 0.1   |
| NZB 17-18       | 17-18            | Ea      | 8            | 10            | 3.8  | 8.1  | 8.8  | —    | 9.0   |
| C3H 16-19       | 16-19            | "       | 8            | 8             | 2.1  | 6.0  | 6.5  | —    | 6.6   |
| Balb/c 15-18    | 15-18            | "       | 8            | 10            | 1.7  | 4.9  | 4.7  | —    | 5.3   |
| C57Bl 16        | 16               | "       | 8            | 6             | 1.6  | 2.5  | 2.4  | —    | 3.5   |
| Newborn Escape: |                  |         |              |               |      |      |      |      |       |
| NZB and B/W     | 2                | BGG     | 8            | 7             | 0.1  | 2.7  | 4.6  | 4.7  | —     |
| B/W 7-9         | 7-9              | "       | 8            | 6             | 2.6  | 2.3  | 3.0  | 4.1  | —     |
| C3H 2           | 2                | "       | 8            | 10            | 0.0  | 0.2  | 0.3  | 0.7  | —     |
| C57Bl 7-9       | 7-9              | "       | 8            | 4             | 0.0  | 0.2  | 1.2  | 1.2  | —     |
| NZB and B/W     | 2                | Ea      | 5            | 8             | 4.5  | 7.1  | 7.5  | 7.5  | —     |
| B/W 7-9         | 7-9              | "       | 5            | 5             | 4.0  | 7.4  | 7.0  | 8.0  | —     |
| C3H 2           | 2                | "       | 5            | 8             | 4.2  | 5.8  | 6.6  | 6.6  | —     |
| C57Bl 7-9       | 7-9              | "       | 5            | 4             | 3.6  | 4.2  | 6.0  | 6.2  | —     |

* Challenge with BGG in Freund's adjuvant at 28 days of age.
† Number of mice surviving at 6-wk bleeding.

Fig. 3. Antibody response curves to challenge with BGG in adjuvant in young (3-wk old) NZB and control strain mice.
24 hr later, 8–10 mg of soluble BGG or Ea was given intraperitoneally. Mice repopulated with either young or old spleen cells and given Ea had antibody titers 14 days postchallenge which were still rising at 35 days (Fig. 6). Mice repopulated with old spleen cells and given BGG showed an identical response, indicating that tolerance was not induced. On the other hand, old mice repopulated with young spleen cells and given BGG made no antibody at 14 days, and showed escape from tolerance between 21 and 35 days. This is the characteristic

| Treatment of mothers | Titer* | No. of animals |
|----------------------|--------|---------------|
| BGG                  | 0.8    | 26            |
| None                 | 4.2    | 18            |

* Hemagglutination titer of 2-month old NZB/NZW F₁ offspring challenged at 1 month with BGG in Freund's adjuvant.

Fig. 4. Partial tolerance in B/W mice and complete tolerance in C3H mice treated from birth with 10–15 mg of soluble BGG biweekly. Each point represents 13–15 mice.

transient tolerance response curve of young animals (Fig. 1), and indicates that tolerance was induced in these older mice after repopulation with young spleen cells. Moreover, these young spleen cells both develop tolerance and escape from tolerance in the recipient mice without benefit of a thymus.

Thymus-Bone Marrow Transfers.—The thymus has been implicated in tolerance induction (9) and is necessary in some systems for the escape from the immunologically tolerant state (10). Bone marrow is the site of origin for antibody-
producing cells which develop competence to certain antigens through synergistic interaction with the thymus (11–14).

Fig. 7 and Table III show the results when 2–3 month old thymectomized and irradiated B/W mice were given young bone marrow-thymus or old bone marrow-thymus combinations. As in the spleen cell transfer system, mice repopulated with the old combination and given BGG had an antibody response curve similar to Ea-treated age-matched controls. The 4 wk antibody titers for both BGG- and Ea-treated mice that were given old cells were lower than those customarily seen in intact older animals, perhaps reflecting a diminished pro-

![Graph showing antibody response over time](image)

**Fig. 5.** Tolerance induction in Balb/c, but not in NZB mice, which were given biofiltered BGG. Each point represents 7–8 mice per treatment group. Antibody titers in NZB mice which received biofiltered BGG were not different from titers in NZB mice which received ultracentrifuged BGG (alone) and results are therefore combined for presentation.

liferative cell response to antigen in this artificial system. Nevertheless, the qualitative lack of tolerance induction characteristic of older NZB mice was clearly present.

In contrast, mice repopulated with the young combination of thymus-bone marrow showed characteristic early tolerance induction followed by rapid loss of unresponsiveness (Fig. 7). Control mice treated with Ea made high antibody titers.

Fig. 8 and Table III show the effect of old bone marrow cells in combination with a young thymus graft. Tolerance to BGG was not induced in spite of the
presence of the young thymus. The slopes of the antibody response curves in the four experimental groups were parallel. Antibody depression was about $2^{1/2}$ log₂ dilutions at 4 wk (3.4/6.2) as compared to $6^{1/2}$ dilutions (1.4/8.0) at 4 wk in mice that were given young marrow–young thymus combinations (Table III). Thus bone marrow cells from older B/W mice appear to contain cells resistant to immunological tolerance which exert their effect, independent of the age of the thymus.

The effect of young bone marrow cells in combination with an old intact thymus gland is shown in Fig. 9 and Table III. Mice having this combination and given BGG responded in the same way as similarly treated mice having both young marrow and young thymus glands; and tolerance was induced. Moreover, the antibody response of mice repopulated with young marrow cells in combination with old thymus glands was markedly suppressed by BGG, not only at 4 wk but also at 6 wk postchallenge (Fig. 9). An identical response was seen with an old irradiated thymus gland in combination with young marrow cells and BGG (see Table III, last combination). Thus bone marrow cells from young B/W mice, like spleen cells of the same age, contain a cell population which can initially become tolerant to BGG, independent of the thymus. How-
ever, the rapid escape from this tolerance seems to depend on the presence of a young thymus. Old thymus tissue, either irradiated or intact, does not allow this early escape to take place.

**DISCUSSION**

These experiments confirm our earlier observations on immunologic hyperactivity and abnormal responses to tolerogens in New Zealand mice (1, 3) and explore the cellular basis for these phenomena. New Zealand mice resist the induction and maintenance of immunologic tolerance to BGG. Rapid escape from tolerance occurs even when BGG is administered in utero or in the first few days of life.

We wish to emphasize, however, that the resistance to tolerance is relative and not absolute. We can maintain partial tolerance by repeated injections of BGG from birth (Fig. 4), and with cyclophosphamide we have induced tolerance in New Zealand mice to sheep erythrocytes and to the synthetic RNA polyinosinic-polycytidylic acid (15). Likewise, tolerance to rabbit γ-globulin

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2 Jacobs, M., and N. Talal. Unpublished observations.
can be achieved by repeated injections or with cyclophosphamide (16). Cerottini et al. have recently reported tolerance to ultracentrifuged HGG in B/W mice, but did not comment on the duration of the tolerant state (17).

Several laboratories have observed a premature immunologic maturation and augmented immune reactivity in New Zealand mice that precedes the development of autoimmunity (1, 2, 18–20). Not all antigens are equally capable of eliciting this hyperresponse (17). Nucleic acids in particular are potent antigens in these mice, inducing or augmenting the otherwise spontaneous formation of anti-DNA and anti-RNA antibodies and leading to accelerated immune complex nephritis (21). Both genetic (22) and viral (23) factors probably play a role in these immunologic responses (21).

Balb/c mice resist tolerance because of a highly efficient phagocytic mechanism which can process trace amounts of aggregated protein (8). Balb/c mice, unlike New Zealand animals, become tolerant to biofiltered BGG (Fig. 5), readily maintain tolerance (Fig. 2), and do not show augmented antibody re-
responses (Fig. 3). We suspected that tolerance resistance in New Zealand mice was due to a lymphoid abnormality and not to hyperphagocytosis.

Repopulation of older, thymectomized, and lethally irradiated B/W mice with young, but not old, spleen cells resulted in tolerance followed by escape, even though the thymus was absent. In another mouse strain, escape from tolerance to BGG was dependent upon the thymus (10, 24). Like old spleen, old thymus plus old bone marrow was resistant to tolerance induction, whereas young thymus plus young bone marrow showed transient tolerance followed by rapid escape.

Moreover, old bone marrow cells, whether combined with young or old thymus, produced a basically adult B/W tolerance response curve (see Fig. 8). This implies that resistance to tolerance induction is already present in 2–3 month old B/W mice and cannot be reversed by the presence of a young thymus. In this respect, the bone marrow population has come to resemble old
B/W spleen cells. We cannot determine whether this unusual property represents an intrinsic abnormality of bone marrow cells, is the result of cellular infiltration, or an effect of the thymus interacting with the bone marrow in the donor prior to transplantation.

By contrast, young bone marrow cells in combination with either young, old unirradiated, or old irradiated thymus were capable of expressing tolerance in the older B/W recipients, unlike old bone marrow cells. This finding again suggests that the bone marrow, like the spleen, undergoes an age-dependent alteration in its response to tolerogens. Nevertheless, the bone marrow seems to be under some thymic regulation since escape from tolerance was delayed if young thymus was not present.

The delay of escape observed with 3–4 month old thymus may reflect an abnormally early loss of thymic regulation of lymphoid proliferation. Substitution of a known nonfunctioning irradiated old thymus in our studies produced results identical to those seen with old nonirradiated thymus alone, suggesting a premature loss of function in the New Zealand thymus. Additional experiments

Fig. 9. Tolerance induction with lack of escape to BGG in thymectomized, irradiated old B/W mice which were given young bone marrow cells and an old thymus graft. Young thymus–young bone marrow response is included for comparison.
with other antigens and normal mouse strains are necessary before this point can be firmly established.

We postulate that in New Zealand mice, during the process of immunological maturation, both bone marrow and thymic function become abnormal. Spleen and bone marrow cell populations of older animals are resistant to tolerance induced by ultracentrifuged BGG. This defect appears over time and may reflect an alteration of lymphoid kinetics or interaction, a change in the membrane or other component of individual lymphocytes, or some other mechanism. Neonatal thymectomy of B/W mice has no effect on the abnormal response to BGG and does not ameliorate the autoimmune disease (25). Prime attention must focus, therefore, on the lymphoid precursors present in the bone marrow. Playfair reported that NZB lymphoid precursors transferred the increased plaque-forming response to sheep erythrocytes characteristic of this strain (26). We suggest that both defective lymphoid precursors and abnormal thymic regulation are present in New Zealand mice and probably contribute to the ultimate emergence of the autoimmune disease.

**SUMMARY**

Newborn, 7-9 day, and 16-18 day old NZB and B/W mice were, unlike older New Zealand mice, rendered tolerant to single doses of 8-10 mg of soluble BGG. After challenge, this tolerance was of short duration and escape occurred rapidly. Age-matched and similarly treated C3H, Balb/c and C57Bl mice did not escape from tolerance. Partial tolerance could be maintained by repeated injections of BGG.

Biofiltration ruled out hyperphagocytosis as an explanation for this resistance to tolerance. Tolerance could be induced in older B/W mice if they were thymectomized, irradiated, and repopulated with young (12-15 day), but not old (2-3 month), spleen or bone marrow cells. Old bone marrow cells gave a non-tolerant response even when combined with young thymic grafts. Young bone marrow gave a tolerant response which was followed by the expected rapid escape only if a young thymus graft was also present. Escape was retarded if old thymus, or old irradiated thymus, was combined with young bone marrow. These results are best explained by abnormalities of both lymphoid precursors and thymic regulation.

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