THYMIC REQUIREMENT FOR CYCLICAL IDIOTYPIC AND RECIPROCAL ANTI-IDIOTYPIC IMMUNE RESPONSES TO A T-INDEPENDENT ANTIGEN*

By GARNETT KELSOE,‡ DALE ISAAK,§ and JAN CERNY

From the Departments of Tropical Public Health and Microbiology, Harvard School of Public Health, Boston, Massachusetts 02115

The cyclical appearance of antibody-forming cells (AFC)[1] in the spleen and peripheral blood following a single antigenic stimulus is a well-documented phenomenon (1). Generally, within the 2-wk period after an antigen stimulus, two peaks of AFC are observed. The first peak occurs at ~4–6 d and the second at 10–13 d (1–8). Both T-independent (2–4) and T-dependent antigens (5–8) are capable of inducing very similar cyclical AFC responses in a wide variety of organisms.

Various explanations for the cyclic production of AFC have been offered including: competition between free antibody and AFC precursors for persistent antigen (2, 6); sequential expression of various classes of antigen-specific immunoglobulin (5); the release of antigen by macrophages (2); regulation of AFC formation that is complement dependent (3); synchronous recruitment of memory B cells that is regulated by highly localized levels of specific antibody (7); and regulator T cells (8).

Recently, results from our laboratory have indicated that the cyclical appearance of splenic plaque-forming cells (PFC) may be the result of cell-to-cell interactions based upon idiotypic recognition (4). We observed a cyclical production of splenic PFC after a single injection of heat-killed strain R36a *Streptococcus pneumoniae* (Pn) in BALB/c mice. Concurrent with the anti-Pn response was a cyclical and reciprocal expansion (or activation) of the clone(s) recognizing the dominant idiotype of the myeloma protein secreted by the TEPC-15 plasmacytoma (T-15) of the Pn-stimulated lymphocytes.

As both T and B cells are known to participate in anti-idiotypic responses, we

* Supported in part by American Cancer Society grant IM-35 and by U. S. Public Health Service grant RO1-CA-14922, and contract NO1-CB-64035 from the National Cancer Institute.

‡ Recipient of a Rockefeller Foundation Fellowship. Present address: Institute for Genetics, University of Cologne, Cologne, Federal Republic of Germany.

§ Supported by U. S. Public Health Service Fellowship 1 F32 CA 06122-01 from the National Cancer Institute. Present address: Department of Microbiology and Immunology, Kirksville College of Osteopathic Medicine, Kirksville, Mo.

[1] *Abbreviations used in this paper:* AFC, antibody-forming cells; BBS, borate-buffered saline; FCS, fetal calf serum; HA, hemagglutination; HBSS, Hanks' balanced salt solution; LM, phenotypically normal littermates of nude mice; M-315, the idiotype of the myeloma protein secreted by the MOPC-315 plasmacytoma; M-603, the idiotype of the myeloma protein secreted by the McPC-603 plasmacytoma; PFC, plaque-forming cells; Pn, *Streptococcus pneumoniae*, strain R36a; PnC, cell wall polysaccharide purified from *S. pneumoniae*; PnC-SRBC, sheep erythrocytes coated with PnC; TG-nude, thymus gland-reconstituted nude mice; T-15, the dominant idiotype of the myeloma protein secreted by the TEPC-15 plasmacytoma; TNP, trinitrophenyl; TNP-SRBC, TNP-substituted sheep erythrocytes; saline, 0.15 M NaCl; SRBC, sheep erythrocytes.

wished to functionally analyze the role of the two cell populations in terms of the cyclical immune response. Thus, we studied both the idiotypic (antigen reactive) and anti-idiotypic immune responses in normal, athymic (nude), and thymus-grafted nude mice after a single antigenic challenge with Pn. We could compare, by utilizing normal and nude mice, the effects of direct antibody feedback mechanisms and those regulatory mechanisms requiring T cells upon the cyclical course of both the idiotypic and anti-idiotypic immune response.

This study demonstrates that both the cyclical idiotypic (antigen specific) and anti-idiotypic immune responses are thymus dependent in BALB/c mice. Nude mice (backcrossed onto the BALB/c genetic background) respond with only a single peak of anti-Pn PFC that is equal to the initial BALB/c response. In both groups of mice, the T-15 clone is dominant and circulating anti-Pn antibody titers are similar. The reciprocal anti-idiotypic response does not occur in nude mice but thymic reconstitution of nude mice restores the cyclical idiotypic and anti-idiotypic responses. These results support the concept that the cyclical immune response is a product of regulatory interactions between idiotypic and anti-idiotypic lymphocyte clones and that T cells are required for the cyclical responses after immunization with a T-independent antigen.

Materials and Methods

Animals. BALB/c mice were purchased from Charles River Breeding Laboratories (Wilmington, Mass.). Congenitally athymic, nude (nu/nu) mice with the BALB/c genetic background and their phenotypically normal littermates (LM) (+/nu or +/+) were obtained either through a National Cancer Institute contract with Charles River Breeding Laboratories or were from our own colony founded from mice provided by Dr. Norman Reed, Montana State University, Bozeman, Mont. The latter are produced by heterozygous parents derived from the ninth backcross on the BALB/c strain. Only mice from the same source were used in an individual experiment (Charles River Breeding Laboratories, exp. 1 and 2; and mice from our colony, exp. 3).

Thymic reconstitution of nude mice was accomplished by implanting a single neonatal BALB/c thymus beneath the capsule of each of the recipient's kidneys (thymus gland-reconstituted nude [TG-nude] mice) as described by Dukor et al (9). All TG-nude mice were examined for evidence of a successful thymic graft at the time of the PFC assay. TG-nude mice were rested at least 4 wk after thymic implantation and used within 6 wk post-implantation. All mice within a single experiment were age-matched to ~ ± 3 wk. Mice of both sexes were used.

Antigen and Immunizations. Pn was grown in Todd-Hewitt broth and harvested during exponential growth according to the method of Liu and Gotschlich (10). The recovered bacteria were washed four times in 0.15 M NaCl (saline) and lyophilized in sterile vials (Wheaton Scientific Div., Wheaton Industries, Millville, N. J.; catalog No. 224822). A bacterial vaccine was prepared by reconstituting the contents of a vial with sterile distilled water and heating the contents to 60°C for 45 min. No viable bacteria could be demonstrated after this treatment. The suspension was then adjusted to a concentration of 100 μg bacterial lyophylate/ml saline. This Pn vaccine was stored at 4°C for the duration of an experiment.

The time-course of the idiotypic (T-15) and anti-idiotypic (anti-T-15) clonal responses after stimulation by Pn was visualized by the sequential immunization of groups (three to four) of mice. Daily, for 14 d, a group of mice was immunized by an intraperitoneal injection of 20 μg of Pn vaccine. On the 15th d, all immunized mice and a control group of comparable (normal, nude, or TG-nude) unimmunized mice were killed and their spleens and sera assayed as described below. Thus each group represented a unique stage of the anti-Pn response.

Preparation of the Cell Wall Polysaccharide of S. pneumoniae (PnC)-coated Sheep Erythrocytes. PnC was extracted according to the procedure of Liu and Gotschlich (10) with the exception that sodium deoxycholate was used at a concentration of 1.0% and that purification was halted after

ether extraction and dialysis against cold, distilled water. This dialyzate was lyophilized and stored at −20°C.

Sheep erythrocytes (SRBC), (Colorado Serum Co., Denver, Colo.) were coated with the recovered PnC by a procedure similar to that for bacterial lipopolysaccharide described by Möller (11). Briefly, PnC was dissolved in saline to a concentration of 0.1–0.2 mg/ml. This solution was heated in a boiling water bath for ~30 min, briefly cooled, and then added to packed, washed (three times in saline) SRBC at a ratio of 0.5 ml PnC solution:0.1 ml packed SRBC. The cells were then gently mixed with a Pasteur pipette and allowed to incubate with the PnC solution for 30 min at 37°C. The cells were washed twice in a large excess (20- to 50-fold) of saline and finally in Hanks' balanced salts solution (HBSS), pH 7.1.

The success of PnC coating was determined by specific hemagglutination (HA) using clarified ascitic fluid from BALB/c mice bearing, intraperitoneally, the TEPC-15 plasmacytoma. The ascitic fluid did not agglutinate normal SRBC and the hemagglutination titer against PnC-coated SRBC (PnC-SRBC) was always ≥1:100,000.

Source and Iodination of Myeloma Proteins. The TEPC-15, MOPC-315, and McPC-603 BALB/c plasmacytoma lines were obtained from Litton Bionetics (Kensington, Md.) under a National Cancer Institute contract. Each tumor was maintained by subcutaneous passage in BALB/c mice. Ascites fluid containing the relevant myeloma protein was obtained by injecting mice intraperitoneally with 0.5 ml of mineral oil (Diamond Drug Incorporated, Westhaven, Conn.) at least 1 wk before an intraperitoneal inoculation of tumor cells. The resulting ascitic fluid was recovered, pooled, and clarified by centrifugation. The proteins contained in each ascitic fluid were twice precipitated by the addition of an equal volume of saturated $(NH_4)_2SO_4$. This precipitate was dissolved in a small amount of water and extensively dialyzed against borate-buffered (0.1 M) saline (BBS), pH 8.0.

The dialyzate was then further purified by gel filtration on a Sephadex G-200 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) column equilibrated with BBS. The eluate was monitored for absorption at 280 nm and for the ability to agglutinate antigen-coated SRBC. A protein peak corresponding to the void volume of the Sephadex G-200 column also contained most of the HA activity. The recovered TEPC-15 (IgA, κ; anti-phosphorylcholine) and McPC-603 (IgA, κ; anti-phosphorylcholine) myeloma proteins agglutinated PnC-SRBC but not trinitrophenyl (TNP)-substituted (TNP-SRBC) (12) or normal SRBC. The MOPC-315 myeloma protein (IgA, λ_2; anti-nitrophenyl) agglutinated only TNP-SRBC. The recovered purified myeloma proteins were concentrated to ~20 mg protein/ml BBS and stored at −60°C.

Radio-iodination of purified myeloma proteins was accomplished via the *N*-hydroxysuccinimide ester of mono-iodinated (^{125}I) *p*-hydroxyphenylpropionic acid (13), obtained from New England Nuclear (Boston, Mass.). Iodination was carried out generally according to the method of Bolton and Hunter (14). Briefly, a volume (≤500 μl) of benzene containing ≃1.0 mCi of ^{125}I (≃1,500 Ci/mmol) was transferred to a chilled (~5°C) vial and the benzene evaporated with a stream of dry N_2 gas. The vial was then cooled to 0°C and 5 mg of myeloma protein added (10 mg/ml BBS). The reaction was allowed to continue for 1.5–2 h at 0°C with continuous stirring. At this time, 0.5 ml of 0.2 M glycine (in BBS) was added, destroying any unreacted iodination reagent.

The iodinated myeloma proteins were separated from any contaminating glycine (and/or glycine conjugates) and 3-(4-hydroxyphenyl) propionic acid by gel filtration over Sephadex G-50. Columns were equilibrated with 0.05 M phosphate buffer, pH 7.5, and ≃10 mg of bovine serum albumin was washed through before the addition of the iodination reaction products. Two peaks of radioactivity were recovered, the first coinciding with the void volume of the column. This first peak was dialyzed against continuously flowing phosphate-buffered saline for 48 h at 4°C. The sp act of the recovered dialyzate was ≃6–8 × 10^4 cpm/μg protein and ≥93% of this activity was precipitated by trichloroacetic acid. The ability of the radiolabeled myeloma proteins to hemagglutinate the appropriate antigen-coupled SRBC was the equivalent of unlabeled proteins.

Serum Antibody Assay. Mice were killed by decapitation and ~0.4 ml of blood was collected from each mouse. The collected blood was allowed to clot overnight at 4°C. Serum was then harvested from each tube, heat inactivated, and absorbed with SRBC. Finally, a 50-μl serum

sample was tested for its capacity to agglutinate PnC-SRBC. The initial dilution of each serum was 1:4 and all titrations were performed in duplicate, using microtiter plates (Cooke Engineering Co., Alexandria, Va.).

Preparation of Lymphocytes. Immediately after blood collection, the spleen was removed into a dish containing ice-cold HBSS. A suspension of splenocytes was then made by pressing the spleen through a stainless steel screen. This suspension was transferred to a conical-bottomed tube. Cell clusters and noncellular debris were removed by allowing the spleen cell suspensions to sediment briefly and then transferring the supernates to a second tube. These cell suspensions were then washed three times in cold HBSS containing 2% fetal calf serum (FCS) (Microbiological Associates). The washed cells were resuspended in 5 ml of HBSS containing 2% FCS, the numbers of viable lymphocytes/ml determined by trypan blue exclusion, and the volume of each cell suspension adjusted to a final concentration of 10^7 viable lymphocytes/ml.

PFC Assay. Aliquots of these suspensions were used to determine the numbers of anti-Pn PFC/10^6 splenocytes. A modified hemolytic plaque assay in agarose (15) was employed utilizing PnC-SRBC (4). The background of anti-SRBC PFC (routinely $\leq 5/10^6$ splenocytes) was determined and that number subtracted from the anti-PnC-SRBC PFC count. Each determination was performed in duplicate.

Idiotype-binding Assay. Cell-associated anti-idiotypic activity was determined as the ability of an aliquot of 10^6 viable splenocytes to bind specifically the T-15 idiotype. This was accomplished by incubating the cells with either the radiolabeled TEPC-15 or a control myeloma protein (McPC-603 or MOPC-315) and determining the cell-bound activity. Specific T-15 binding was calculated as:

$$\text{T-15 bound (percentage of control)} = \frac{(\text{counts per minute T-15 immunized}) - (\text{counts per minute M-603 immunized})}{(\text{counts per minute T-15 control}) - (\text{counts per minute M-603 control})} \times 100.$$

Specifically, 5 μg of radiolabeled myeloma protein (preliminary experiments indicated that 1 μg of either TEPC-15 or MOPC-315 saturated the binding capacity of 10^6 BALB/c splenocytes [4]) was added to duplicate tubes containing 10^6 splenocytes in ice-cold HBSS containing 2% FCS. The tubes were incubated overnight at 4°C. The cells were then washed four times with cold HBSS containing 10% FCS and the resultant cell pellets counted in a Packard γ-spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The cpm/tube ranged from $\simeq 5 \times 10^3$ to 12×10^3 cpm, representing ~60 ng of bound myeloma protein. The variation between duplicates was routinely ≤12%.

Only a single control (the idiotype of the myeloma protein secreted by the MOPC-315 or the McPC-603 plasma cytoma) idiotype was used in a given experiment. Previous experiments demonstrated that either protein serves equally well as a control (4).

Inhibition of PFC by Heterologous Anti-T-15 Serum. Rabbit anti-T-15 serum was raised by injecting purified TEPC-15 myeloma protein into rabbits. The antiserum was rendered specific by absorption with pooled murine IgM and the purified MOPC-315 myeloma protein insolubilized onto Affi-gel 10 Sepharose beads (Bio-Rad Laboratories, Richmond, Calif.) and by incubation with normal C57BL/6 splenocytes (10^7/ml antiserum). The addition of this antiserum at a 1:500 final dilution inhibited by ≃85% the number of anti-Pn PFC produced by a representative pool of Pn-immunized BALB/c mice. The numbers of either C57BL/6 anti-Pn PFC or BALB/c anti-SRBC-PFC were not inhibited at the same concentration.

Statistical Analyses. In certain cases Student's *t* statistic (two-tailed) was employed to determine if significant differences ($P \leq 0.05$) existed between serial PFC values.

Results

The specificity of the labeled idiotype probes was determined by inhibition studies utilizing unlabeled homologous or heterologous protein competitors (Fig. 1). Addition of a 100-fold excess of unlabeled homologous protein resulted in a >50% decrease in binding of the labeled idiotype. The same excess of heterologous protein resulted in only ≃18% inhibition.

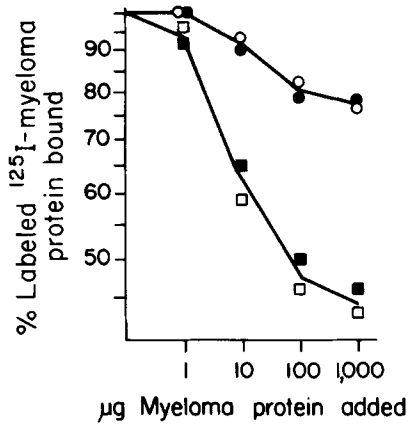

FIG. 1. Inhibition of binding of radiolabeled myeloma proteins on BALB/c spleen cells (10^6) in the presence of increasing amounts of competing unlabeled homologous or heterologous myeloma proteins (abscissa). The amount of cell-bound activity is expressed as the percent of that in the absence of any competitor. ^{125}I-TEPC-15 (1 μg) plus TEPC-15 (□) or plus MOPC-315 (○); ^{125}I-MOPC-315 (1 μg) plus MOPC-315 (■) or plus TEPC-15 (●).

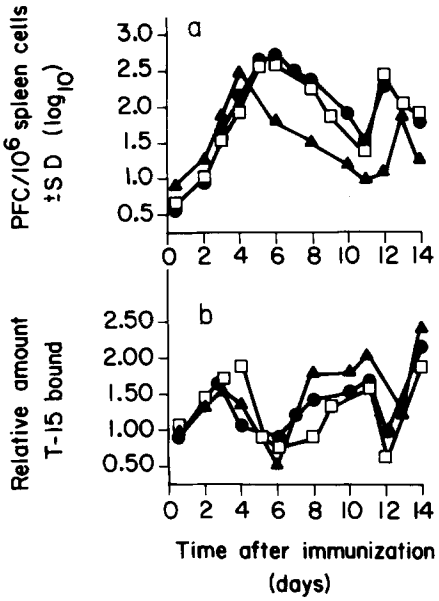

FIG. 2. The kinetics of primary immune response of BALB/c mice to Pn: (a) specific anti-Pn PFC in the spleen; (b) relative changes in binding of ^{125}I-TEPC-15 by splenocytes. Each point represents the mean from a cohort of three to four mice, in three successive experiments (□, ●, and ▲).

The kinetics of both PnC-specific PFC production and relative T-15 binding by spleen cells from Pn-immunized BALB/c mice were determined in three successive experiments (Fig. 2). The accumulation of specific PFC after a single injection of vaccine followed a cyclical pattern over a 14-d period; an initial relatively broad peak (≃400 PFC/10^6 splenocytes) occurred on day 4–6 declining variably thereafter until a second sharper peak (≃150 PFC/10^6 splenocytes) appeared on day 12–13 (Fig. 2a). The pattern of T-15 binding was also cyclical, with heights of binding activity

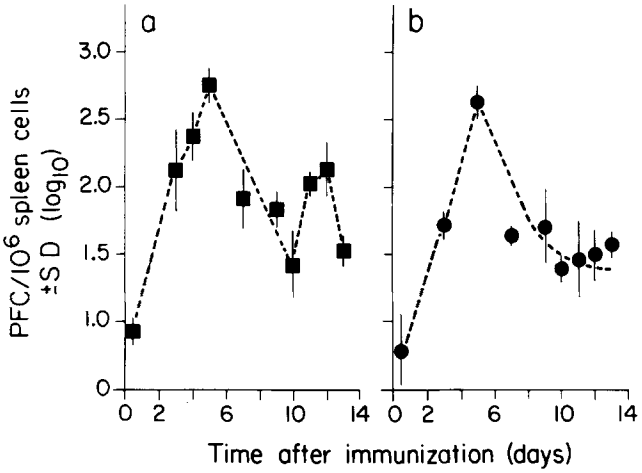

FIG. 3. The kinetics of primary anti-Pn PFC response in the spleen of: (a) BALB/c mice (■), and (b) nude mice (●). Each point represents the mean from a cohort of three to four mice with standard deviations (SD) indicated by the vertical bar.

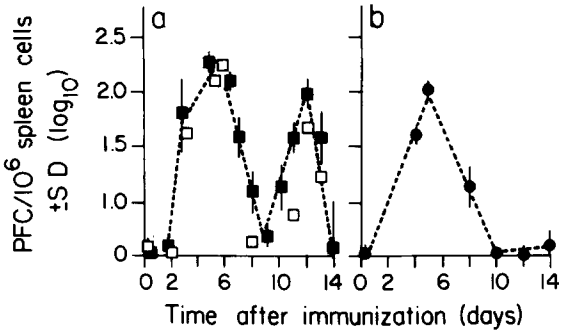

FIG. 4. The kinetics of Pn-specific PFC responses in the spleens of: (a) BALB/c mice (■) and LM (□); and (b) nude mice (●). Each point represents the mean from a cohort of three to four mice with standard deviations (SD) indicated by the vertical bar.

occurring on day 2–4, day 10–11, and day 14 (Fig. 2b). This reciprocal out-of-phase relationship between the curves of Pn-specific PFC and T-15 binding is typical of the BALB/c anti-Pn immune response (4).

In contrast, the kinetics of splenic anti-Pn production in thymus-deficient, nude mice immunized with Pn vaccine was not cyclical (Fig. 3b). Instead, a first peak of specific PFC reached on day 5 (445 ± 170 PFC/10^6 splenocytes) was followed by a decline and then a plateau during which the PFC values ranged between 25 ± 13 (day 10) and 40 ± 15 (day 13). No significant second peak of anti-Pn response occurred. The control group of euthymic BALB/c mice displayed a typical biphasic response (550 ± 160 PFC, day 5, and 225 ± 24 PFC, day 12) (Fig. 3a). Similar results were obtained in another experiment comparing nude mice with both LM and BALB/c mice (Fig. 4). The anti-Pn PFC responses of euthymic mice were essentially the same, with a first peak on day 5–6 followed by a decline to near background by day 10 and a second peak again on day 12 (Fig. 4a). Nude mice, however, responded by only one round of PFC culminating on day 5 (140 ± 20 PFC/10^6 splenocytes) (Fig. 4b).

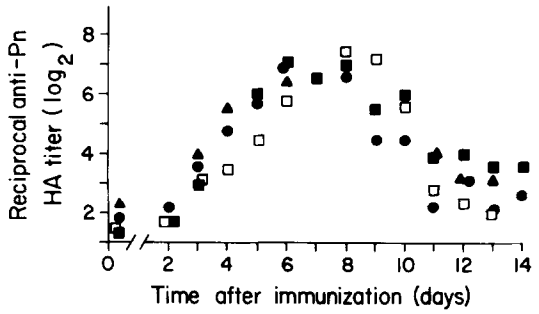

FIG. 5. Anti-Pn antibody determined by passive HA in the serum of mice immunized with Pn vaccine (20 μg intraperitoneally, on day 0). Each point represents the mean titer in a cohort of three to four mice bled and killed on the indicated day after immunization: BALB/c (■), nude (●), LM (□), and TG-nude (▲) mice.

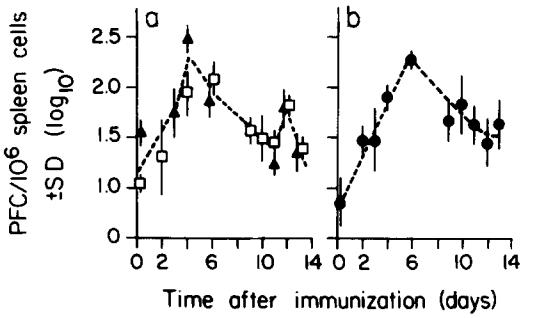

FIG. 6. The kinetics of Pn-specific PFC responses in the spleens of: (a) LM (□) and TG-nude mice (▲); (b) nude mice (●). Each point is the mean ± SD from a cohort of four mice.

It should be noted that the circulating antibody response to Pn was indistinguishable between BALB/c, nude, LM, and TG-nude mice as measured by Pn-specific passive HA. Typically, the serum anti-Pn antibody titers would peak at day 8 and thereafter decline. By day 14, all mice showed significantly decreased levels (20–30% of maximum values) of Pn-specific serum antibody (Fig. 5).

In all mice, the T-15 component of the anti-Pn response was dominant as measured by PFC inhibition. Pooled cells of representative BALB/c, nude, and LM mice were equally inhibited (75–85%) by a specific rabbit anti-T-15 serum. In cases where more than a single anti-Pn PFC peak occurred, both were predominantly T-15 positive.

The experiments shown in Figs. 3 and 4 were repeated using euthymic LM, nude mice, and TG-nude. Again, the splenic anti-Pn PFC activity of LM mice was biphasic with an initial peak on day 5–6 (140 ± 50 PFC/10^6 splenocytes) followed by a second peak on day 12 (80 ± 20 PFC/10^6 splenocytes) (Fig. 6a). A biphasic pattern was not apparent in nude mice (Fig. 6b). In this experiment, nude mice reached an initial anti-Pn peak on day 5 (180 ± 30 PFC/10^6 splenocytes) that declined significantly by day 9. Anti-Pn values for day 9–14 ranged from a high of ≃70 (day 10) to a low of ≃30 (day 12). No significant peak of anti-Pn activity occurred. However, when nude mice were grafted with genetically compatible BALB/c thymus (TG-nude mice) and immunized with Pn, the distinctive biphasic kinetics of PFC production returned, with peaks on days 4 (280 ± 40 PFC/10^6 splenocytes) and 12 (75 ± 35 PFC/10^6 splenocytes) (Fig. 6a).

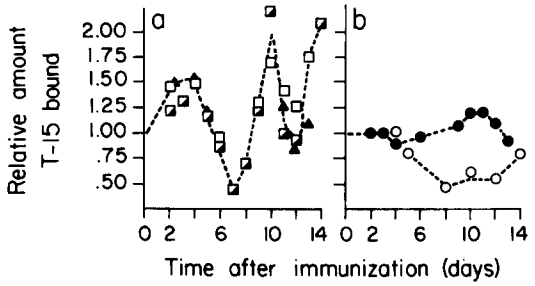

FIG. 7. Relative changes in binding of ^{125}I-TEPC-15 by spleen cells during the anti-Pn antibody response. Values are based upon the ratio of specific T-15 binding (Materials and Methods) between immunized and nonimmunized mice. (a) BALB/c mice (◪), LM (□) and TG-nude mice (▲); (b) nude mice, exp. 1 (○) and exp. 2 (●).

The changes in T-15 binding by splenic lymphocytes also differed between normal and athymic mice. The data shown in Fig. 7 bear on the Pn response in Figs. 4 and 6. BALB/c, LM, and TG-nude mice showed patterns of T-15 binding that were virtually identical (Fig. 7 a) and typical of the BALB/c anti-Pn immune response (4). Peaks of T-15 binding occurred at day 3–4 and day 10–11. These expansions are the reciprocal of the corresponding PFC kinetics. Conversely, T-15 binding in nude mice either declined and remained depressed (Fig. 7 b, exp. 1) or did not seem significantly affected by the ongoing anti-Pn response (exp. 2). The changes that did occur were not in phase with the T-15 binding oscillations typical of the thymus-associated BALB/c anti-Pn immune response.

Discussion

Our experiments demonstrate that the repeated (biphasic) appearance of anti-Pn antibody (IgM) PFC in mice immunized with a single injection of Pn vaccine depends on the presence of the thymus. Although Pn vaccine induces in nude mice an initial peak of T-15-bearing PFC and levels of circulating anti-Pn antibody indistinguishable from that occurring in normal mice, a biphasic PFC response is absent. Thymic reconstitution restores the cyclical immune response. Thus, at least a portion of the anti-Pn response seems to be thymus-dependent. Although our experiments cannot rule out an effect of antibody that is mediated by T cells, it seems clear that serum antibody does not affect the PFC precursors either directly or via competitive interactions with antigen in such a way as to produce a biphasic anti-Pn response.

The immunization of BALB/c mice with Pn vaccine is accompanied by expansion or activation of cells binding the dominant idiotype, T-15, of the Pn-reactive B cells. Appropriate control experiments were performed to demonstrate that the increased binding of ^{125}I-TEPC-15 is indeed specific for the idiotopic determinant and thus reflects the amount of cell-associated T-15 receptors (i.e., anti-idiotypic cells). The increased T-15-binding activity seen in BALB/c mice was completely absent in Pn-immunized nude mice. The latter could not be a result of a lack of idiotypic stimulus because the initial response of T-15-positive, anti-Pn PFC in nude mice is equivalent to that in either BALB/c or LM mice. Furthermore, implantation of a thymus in nude mice restores their ability to express the anti-idiotypic cellular activity.

Thus it would seem that although a sufficient idiotypic stimulus is present in nude mice, a component necessary for the anti-idiotypic response is not. Ostensibly, this

component is anti-idiotypic T cells. That both cyclical PFC production and the anti-idiotypic response are missing in thymusless mice strongly implies that the cyclical nature of the anti-Pn response is causally linked with the reciprocal anti-idiotypic response. This is entirely consistent with the finding of Romball and Weigle (8) that T cell and not B cell mitogens are capable of abrogating the cyclical immune response.

Cycling has been noted in various species with antigens ranging from haptens (16) to bacterial antigen (2) and alloantigens (17), and in both T-independent and T-dependent antibody responses (2–8), as well as cell-mediated responses (18, 19). The cycling is independent of the immunization route and of the serum antibody levels (1–8). What is most remarkable is that the peaks of AFC appear almost exactly every 6–10 d regardless of the antigen or animal species. This constancy leads us to believe that the cyclical immune response is the expression of a fundamental cybernetic system for immunological regulation. Viewed in this light, our data appear to be a significant demonstration of the idiotype-specific, regulatory equilibrium originally postulated by Jerne (20–22).

Numerous studies have demonstrated that the capacity for idiotype-specific immune regulation exists (23–29) most often by demonstrating idiotype-specific help, suppression, or activation. Our data are similar in that we demonstrate that specific activation of the anti-T-15 clone(s) occurs during the BALB/c anti-Pn immune response. More importantly, our data indicate that the interaction between idiotypic and anti-idiotypic clones is thymus-dependent, mutually reciprocal, and continuing. There is no reason to expect that this cycling ends after 14 d. Indeed, cyclical AFC production has been shown to occur for at least 50 d (30) and regularly periodic fluctuation in antibody production has been measured over a period of years (31). The longevity of these cyclical phenomena seemingly preclude a role for exogenous antigen. Instead, it is likely that the basis for continuing immune periodicity is entirely internal.

Recently, Eichmann et al. (32) have demonstrated that under the appropriate conditions, anti-idiotypic T-helper cells can induce B-memory cells to become AFC, even in the absence of antigen. It is conceivable that periodic waves of anti-idiotypic helper cell activity could give rise to the long-lived cyclical immune response. The cyclical immune response then, may be visualized as a dynamic regulatory equilibrium that continuously balances idiotypic and anti-idiotypic cell populations via positive and negative regulatory interactions.

The effectors of this regulation appear to be T cells. Thus, the cyclical immune response is thymus-dependent. However, since Pn is a T-independent antigen, it is likely that both T and B cells may induce those regulatory events leading to the cyclical immune response. That primed B cells do have the capacity to induce T-cell-mediated suppression has been demonstrated in other systems (33).

Finally, as it is known that the cyclical immune response may be abrogated by the administration of passive specific antibody (30), the concept of anti-idiotypic regulation may complement previously proposed mechanisms of immune regulation, in particular the antibody feedback effect. Even in those situations where the steady level of circulating antibody ostensibly bears no relationship to the cycling appearance of idiotypic or anti-idiotypic cells (4, 8, and this paper), significant cell-antibody interactions might still occur *in situ*. Indeed, preliminary results indicate that T-15 binding increases after mild trypsinization and subsequent in vitro incubation of splenocytes from Pn-immunized BALB/c mice (G. Kelsoe and J. Cerny. Unpublished results.).

Summary

The role of the thymus in the cyclical appearance of the dominant idiotype of the myeloma protein secreted by the TEPC-15 plasmacytoma (T-15)-bearing plaque-forming cells (PFC) and anti-idiotypic cells (i.e., cells with receptors for T-15) in the spleen during a primary response to the phosphorylcholine determinant of *Streptococcus pneumoniae*, strain R36a (Pn) was studied using normal mice, thymus-deficient nude mice, and thymus gland-grafted nude mice (TG-nude). The nude mice and their phenotypically normal littermates (LM) were backcrossed on the BALB/c genetic background.

The kinetics of the anti-Pn PFC response of BALB/c inbred mice, littermates of nude mice, and TG-nude mice were essentially the same. There was an initial peak on day 5–6 followed by a decline to near background, and then a second peak on day 12. In nude mice, the first peak of anti-Pn PFC (day 5) was comparable in magnitude to that of mice with an intact thymus; however, there was no second peak. In contrast to the cellular response measured at the level of PFC, the serum antibody response to Pn (assayed by passive hemagglutination of sheep erythrocytes coated with Pn polysaccharide) was comparable in all groups of mice and did not show a measurable oscillation.

The anti-idiotypic cellular activity was determined by the ability of spleen cells to bind radiolabeled (^{125}I) TEPC-15 myeloma protein (IgA, κ) which carries an idiotypic determinant indistinguishable from that of most anti-phosphorylcholine antibodies in BALB/c mice. Binding of radiolabeled McPC-603 (IgA, κ) and MOPC-315 (IgA, λ_2) myeloma proteins (which lack the T-15 idiotypic determinant) served as controls. The changes in T-15 binding by splenic lymphocytes following the Pn immunization differed between normal and athymic mice. BALB/c, LM, and TG-nude mice showed a biphasic pattern with peaks at days 3–4 and 10–11 that was nearly reciprocal to the PFC curve. On the other hand, T-15 binding in nude mice either declined and remained depressed or was not affected by the ongoing anti-Pn response.

These observations demonstrate that mature T cells are required for cyclical idiotypic and anti-idiotypic responses to immunization with a T-independent antigen and suggest that the cyclical immune response may result from an interaction between idiotypic and anti-idiotypic cell clones.

Received for publication 23 July 1979 and in revised form 16 October 1979.

References

1. Weigle, W. O. 1975. Cyclical production of antibody as a regulatory mechanism in the immune response. *Adv. Immunol.* **21**:87.
2. Britton, S., and G. Möller. 1968. Regulation of antibody synthesis to *Escherichia coli* endotoxin. I. Suppressive effect of endogenously produced and passively transferred antibodies. *J. Immunol.* **100**:1326.
3. Nielson, K. H., and R. G. White. 1974. Effect of host decomplementation on homeostatis of antibody production in fowl. *Nature (Lond.).* **250**:234.
4. Kelsoe, G., and J. Cerny. 1979. Reciprocal expansions of idiotypic and anti-idiotypic clones following antigen stimulation. *Nature (Lond.).* **279**:333.
5. Wortis, H. H., R. B. Taylor, and D. W. Dresser. 1966. Antibody production studied by means of the LHG assay. I. The splenic response of CBA mice to sheep erythrocytes. *Immunology.* **11**:603.

6. Sell, S., A. B. Park, and A. A. Nordin. 1970. Immunoglobulin classes of antibody-forming cells in mice. I. Localized hemolysis-in-agar plaque-forming cells belonging to five immunoglobulin classes. *J. Immunol.* **104**:483.
7. Romball, C. G., and W. O. Weigle. 1973. A cyclical appearance of antibody-producing cells after a single injection of serum protein antigen. *J. Exp. Med.* **138**:1426.
8. Romball, C. G., and W. O. Weigle. 1976. Modulation of regulatory mechanisms operative in the cyclical production of antibody. *J. Exp. Med.* **143**:497.
9. Dukor, P., J. F. A. P. Miller, W. House, and V. Allman. 1965. Regeneration of thymus grafts. II. Histological and cytological aspects. *Transplantation (Baltimore).* **3**:639.
10. Liu, T.-Y., and E. Gotschlich. 1963. The chemical composition of pneumococcal C-polysaccharide. *J. Biol. Chem.* **238**:1928.
11. Möller, G. 1965. 19S antibody production against soluble lipopolysaccharide antigens by individual lymphoid cells *in vitro*. *Nature (Lond.).* **207**:1166.
12. Kettman, J., and R. W. Dutton. 1970. An *in vitro* primary immune response to TNP-substituted erythrocytes. Response against carrier and hapten. *J. Immunol.* **104**:1558.
13. Rudinger, J., and U. Ruegg. 1973. Preparation of N-succinimidyl 3-(4-hydroxyphenyl) proprionate. *Biochem. J.* **133**:538.
14. Bolton, A. E., and W. M. Hunter. 1973. The labeling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing alcylating agent. Application to radioimmunoassay. *Biochem. J.* **133**:529.
15. Mosier, D. E. 1969. Cell interactions in the primary immune response *in vitro*: a requirement for specific cell clusters. *J. Exp. Med.* **129**:351.
16. Bolton, R. W. 1978. Cyclic appearance of antibody producing cells in the peripheral blood in response to a chemically-defined polyglycerophosphate antigen. *Immunol. Commun.* **7**:383.
17. Stimpfling, J. H., and A. Richardson. 1967. Periodic variations of the hemagglutinin response in mice following immunization against sheep red blood cells and alloantigens. *Transplantation (Baltimore).* **5**:1496.
18. McKearn, T. J., F. P. Stuart, and F. W. Fitch. 1974. Anti-idiotypic antibody in rat transplantation immunity. I. Production of anti-idiotypic antibody in animals repeatedly immunized with alloantigens. *J. Immunol.* **113**:1876.
19. Denham, S., C. K. Grant, J. G. Hall, and P. Alexander. 1970. The occurrence of two types of cytotoxic lymphoid cells in mice immunized with allogenic tumor cells. *Transplantation (Baltimore).* **9**:366.
20. Jerne, N. K. 1973. The immune system. *Sci. Am.* **229**(1):52.
21. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris).* **124C**:373.
22. Jerne, N. K. 1975. The immune system. A web of V-domains. *Harvey Lect.* **70**:93.
23. Trenker, E., and R. Riblet. 1975. Induction of antiphosphorylcholine antibody formation by anti-idiotypic antibodies. *J. Exp. Med.* **142**:1121.
24. Black, S. J., G. J. Hämmerling, C. Berek, K. Rajewsky, and K. Eichman. 1976. Idiotypic analysis of lymphocytes *in vitro*. I. Specificity and heterogeneity of B and T lymphocytes reactive with anti-idiotypic antibody. *J. Exp. Med.* **143**:846.
25. Cosenza, H. 1976. Detection of anti-idiotypic reactive cells in the response to phosphorylcholine. *Eur. J. Immunol.* **6**:114.
26. Owen, F. L., S.-T. Ju, and A. Nisonoff. 1977. Presence on idiotype-specific suppressor T cells of receptors that interact with molecules bearing the idiotype. *J. Exp. Med.* **145**:1559.
27. Cazenave, P. A. 1977. Idiotypic-anti-idiotypic regulation of antibody synthesis in rabbits. *Proc. Natl. Acad. Sci. U. S. A.* **74**:5122.
28. Urbain, J., J. D. Rikler, J. D. Franssen, and C. Collignon. 1977. Idiotypic regulation of the immune system by the induction of antibodies against anti-idiotypic antibodies. *Proc. Natl. Acad. Sci. U. S. A.* **74**:5126.
29. Woodland, R., and H. Cantor. 1978. Idiotype-specific T helper cells are required to induce

idiotype positive B memory cells to secrete antibody. *Eur. J. Immunol.* **8**:600.
30. Britton, S., and G. Möller. 1968. Regulation of antibody synthesis against *Escherichia coli* endotoxin. I. Suppressive effect of endogenously produced and passively transferred antibodies. *J. Immunol.* **100**:1326.
31. Rubenstein, P. 1972. Cyclical variations in anti-Rh titer detected by automatic quantitative hemagglutination. *Vox Sang.* **23**:508.
32. Eichmann, K., I. Falk, and K. Rajewsky. 1978. Recognition of idiotypes in lymphocyte interactions. II. Antigen-independent cooperation between T and B lymphocytes that possess similar and complementary idiotypes. *Eur. J. Immunol.* **8**:853.
33. Ward, K., H. Cantor, and A. Nisonoff. 1978. Analysis of the cellular basis of idiotype-specific suppression. *J. Immunol.* **120**:2016.