Ly PHENOTYPE AND MECHANISM OF ACTION OF MOUSE NEONATAL SUPPRESSOR T CELLS

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Antibody production during the neonatal period appears to be under more stringent regulation than during adult life in the mouse. Cultures of neonatal mouse spleen cells are prevented from fully expressing their B-cell functional potential by the activity of suppressor cells. These cells were tentatively identified as T cells on the basis of their sensitivity to high concentrations of anti-Thy 1.2 (anti-θ) serum and complement or their failure to adhere to nylon wool columns (1). T lymphocytes can now be further divided into functional subpopulations on the basis of their surface Ly antigen expression. It has been reported, for example, that helper T cells are Ly 1+, Ly 2,3− while cytotoxic killer T cells are Ly 1−, Ly 2,3+ (2-6). Suppressor T cells found in the adult mouse spleen appear to express only the Ly 2,3 phenotype (7-13). We have therefore attempted to isolate enriched populations of neonatal suppressor cells from mouse spleen and thymus and characterize these cells both for functional activity and surface expression of Ly 1, Ly 2, TL, Thy 1, and H-2 antigens. Cells were also characterized as to size and DNA content by acridine orange staining and automated cytofluorimetry (14). Enrichment of suppressor T cells was achieved by infection of newborn mice (less than 18 h old) with the herpes virus of mice, mouse thymic virus (15-18). The residual T cells, obtained 10-14 days after infection, were enriched for suppressor activity while they were depleted for most other T-cell functions such as helper activity, mixed lymphocyte and cytotoxic T-cell reactivity, and T-cell mitogen responses (16). This mouse thymic virus-resistant T-cell population, which represents about 5% of the normal T-cell number, thus served as a relatively homogenous preparation high in suppressor activity. It was this population which was analyzed in detail for surface phenotype and mechanism of action.

Materials and Methods

Mice. All mice used in these experiments were (C57BL/6N x DBA/2N) F1 (BDF1) obtained from the Small Animal Section, Division of Research Services, National Institutes of Health. Pregnant C57BL/6N females were delivered to our animal facility and the F1 offspring reared in laminar flow cabinets in semi-isolation. Mice of the X-linked mutant CBA/N strain crossed with

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1 Abbreviations used in this paper: aecm, aminoethylcarbamylmethyl; anti-mlg, goat anti-mouse immunoglobulin; anti-θ, anti-Thy 1.2; BDF1, (C57BL/6N x DBA/2N)x; fl, fluorescein; LPS, lipopolysaccharide; PFC, plaque-forming cell; PHA, phytohemagglutinin; TNP, trinitrophenyl; rh, rhodamine.
DBA/2N males were used to breed the (CBA/N × DBA/2)F1 male (defective) and F1 female (normal) mice used in some experiments.

**Culture Conditions.** Single cell suspensions of mouse thymus or spleen were prepared and cultured by using a microculture adaptation of the Mishell and Dutton method as previously described (19). Briefly, a total of 1 × 10^6 cells were cultured in each well of a flat-bottomed microtiter plate (Falcon 3040, Falcon Plastics, Division of BioQuest, Oxnard, Calif.) for periods of 3–4 days in a humidified 5% CO2–95% air atmosphere. Antibody formation against the trinitrophenyl (TNP) determinant was stimulated by the addition of TNP-aecm Ficoll, TNP-lipopolysaccharide (TNP-LPS), or TNP-Brucella abortus. These antigens appear to be thymic-independent in the newborn as well as the adult mouse (D. E. Mosier. Manuscript in preparation) and their preparation has been described previously (20, 21). Direct plaque-forming cells (PFC) were assayed against TNP-modified sheep erythrocytes prepared as described by Rittenberg and Pratt (22). All groups were assayed in triplicate and the data logarithmically transformed to obtain the geometric mean. Data are expressed as the antilog of the geometric mean ×/− the relative standard error (the antilog of the log standard error). Proliferation assays were performed as described previously.

**Virus Injection.** Mouse thymic virus was obtained by serial passage from a stock maintained by Dr. Wallace Rowe, National Institute of Allergy and Infectious Diseases, National Institutes of Health, and donated to us by Dr. Sue Cross. Newborn mice were injected intraperitoneally within 18 h of birth with a 1:10 dilution of a clarified supernate of virus thymus homogenate. Mice were examined when sacrificed 10–14 days later for the gross thymic necrosis associated with virus infection, and any mice not showing this sign were excluded from the experiment.

**Cytotoxicity Assay.** The complement-dependent cytotoxicity assay of Gorer and O’Gorman (23) was used with the modification described by Boyse et al. (24). Equal 0.05-ml columns of: (a) antiserum serially diluted; (b) selected rabbit serum diluted 1:15 in anti-Ly tests (25, 26), or guinea pig serum diluted 1:4 in anti-TL, -Thy 1, and H-2 tests (both complement sources were selected for low cytotoxicity against mouse thymocytes and high complement activity); and (c) cells suspended in medium 199 (Hank's balanced salt solution base) at 5 × 10^6/ml were incubated at 37°C for 45 min. Cell viability was assessed by trypan blue exclusion.

**Antisera and Immunofluorescence Reagents.** The anti-Ly, anti-TL, and anti-Thy 1.2 sera used in this study were obtained from Doctors E. A. Boyse and F.-W. Shen and details of their specificity are presented elsewhere.2 The anti-H-2 b reagent was obtained from BALB/c AnN female mice which had received both skin grafts and peripheral lymphoid cell suspensions from (BALB/c × C57BL/6N)F1 or C57BL/6 female mice. At the dilutions used in these studies, the antiserum does not react with BALB/c, NZB, or B10.D2 mouse thymocytes. The anti-Thy 1.2 serum used for bulk killing of neonatal spleen cell suspensions was a conventional AKR/J anti-C3H/J thymocyte reagent obtained in the ascites form from Dr. I. Zitron and used after absorption with AKR thymocytes and ultracentrifugation at 100,000 g for 20 min.

The immunofluorescence assays employed a polyvalent goat antiserum to mouse immunoglobulin. Antibody from this antiserum was purified by affinity chromatography on a column containing equal amounts of γ1, γ2m, and γm mouse myeloma proteins. The purified antibody reacted in double diffusion gels with all classes of immunoglobulins and with κ but not λ chains. Goat antibodies to mouse immunoglobulins (anti-mlg) were coupled to fluorescein by reaction with fluorescein isothiocyanate to achieve a molar ratio of fluorescein to protein of 2.5–5.7. Goat anti-mlg were conjugated with rhodamine by reaction with tetramethylrhodamine isothiocyanate isomer R (lot FS EHRT 12, BBL) to achieve a rhodamine:protein molar ratio of 3.0. Unconjugated fluorescein or rhodamine were removed by passage over Sephadex G-25 equilibrated with 0.15 M NaCl, pH 7.4. (Pharmacia Fine Chemicals, Piscataway, N. J.) Rhodamine-anti-mlg also was passed over activated charcoal. These two fluorescent reagents are designated fl-anti-mlg and rh-anti-mlg, respectively.

**Immunofluorescence Assay.** The immunofluorescence test used with the antisera to Ly determinants and the details of the antisera specificity are reported elsewhere. A cell suspension containing 1 × 10^6 cells was prepared in Hanks' MEM containing 10% heat-inactivated fetal calf serum and 0.1% sodium azide, and incubated with the appropriate antisera for 30 min on ice. The
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TABLE I
Mitogen Responses of Normal or Mouse Thymic Virus-Infected 11-Day Old BDF, Thymus and Spleen Cells

|                  | Geometric mean cpm, ×± relative SE |
|------------------|-------------------------------------|
|                  | Mitogen: 0 | PHA | Con A* | LPS         |
|                  |            |     |        |             |
| Cells:           |            |     |        |             |
| Normal thymus    | 182        | 1,604 | 33,074 | 1,586       |
|                  | (1.25)     | (1.11) | (1.08) | (1.11)      |
| Virus-infected thymus | 314      | 7,041 | 59,520 | 828         |
|                  | (1.02)     | (1.11) | (1.20) | (1.17)      |
| Normal spleen    | 904        | 6,422 | 29,547 | 5,126       |
|                  | (1.28)     | (1.18) | (1.17) | (1.02)      |
| Virus-infected spleen | 1,169 | 894  | 4,406  | 37,533      |
|                  | (1.15)     | (1.12) | (1.08) | (1.05)      |

* Con A, concanavalin A.

Results

Neonatal Infection with Mouse Thymic Virus Spares Suppressor Cell Activity in Thymus and Spleen. Neonatal infection of BALB/c mice with mouse thymic virus previously has been shown to decrease most, but not all, T-cell functions within 1 wk of infection (16). Among the functions resistant to the effects of viral infection are phytomitogen responsiveness in the thymus (but not the spleen) (16), the induction of T-cell-mediated suppression of pneumococcal polysaccharide responses (18), and the ability to synergize with effector cells in the graft-versus-host reaction (17). Newborn BDF, mice were injected within 18 h of birth with mouse thymic virus. 10–16 days later, residual cells from their thymuses and spleens were recovered. Thymuses from virus-infected mice yielded 5–10% of the number of cells recovered from uninfected age-matched controls while spleen cell recovery was about 50% of normal. To confirm the differential effect of mouse thymic virus on thymus and spleen T cells, recovered cells were stimulated with Phytohemagglutinin (PHA), Con A, or LPS and [3H]thymidine incorporation determined after 72 h of culture. As shown in Table I, the virus infection had moderately increased the response of residual thymocytes to PHA and Con A, but residual spleen cells had much reduced or absent proliferative responses to both mitogens. The response of splenic B cells to LPS was increased in the cells from viral-infected mice, but the magnitude of the increase was not usually as great as in this particular experiment. Cells from the same group of animals were mixed with graded numbers of adult BDF, spleen cells and the
mixture of cells stimulated with TNP-Ficoll, a thymus-independent antigen. Over the range of cell numbers employed, the number of adult spleen cells cultured was directly and linearly related ($r = 0.97$ in three replicate experiments) to the numbers of PFC's generated after 4 days of culture. Fig. 1 shows the results of adding spleen cells from 10-day old normal or virus-infected BDF₁ mice to adult BDF₁ spleen cells with the total cell number per microculture held constant at $1 \times 10^6$. The ability of spleen cells from the virus-infected mice to interfere with the B-cell triggering induced by TNP-Ficoll was even greater than that of normal 10-day old spleen cells. That this suppression was a result of residual T cells and not a direct effect of mouse thymic virus was shown by demonstrating that: (a) the suppression was still sensitive to treatment of spleen cells with undiluted anti-$\theta$ serum and complement, and (b) direct addition of mouse thymic virus preparation or supernates of spleen cells from infected mice were without suppressive effects (data not shown). The thymocytes which survive mouse thymic virus infection also were shown to be more suppressive of B-cell activation than age-matched control thymocytes (Table II).
This subpopulation of virus-resistant T cells therefore seems to be increased in suppressor activity while previous studies have shown it to be devoid of most other T-cell functions. The thymus and spleen cells of neonatally mouse thymic virus-infected BDF1 mice were chosen on this basis as a relatively homogenous population for analysis of cell surface phenotype and morphology.

**Ly 1, Ly 2, Thy 1, TL, and H-2 Phenotype of Mouse Thymic Virus-Resistant Thymocytes.** The population of cells obtained from the thymuses of BDF1 mice injected as neonates with mouse thymic virus (and demonstrated in parallel experiments to be enriched for suppressor activity) were tested both by cytotoxicity and immunofluorescence assays for expression of mouse cell surface alloantigens. In each experiment, cytotoxicity assays or immunofluorescence labeling was performed in parallel on age-matched normal thymocytes as well as mouse thymic virus-resistant thymocytes. Susceptibility of the two cell populations to killing or staining by anti-Ly antisera first was assessed. Since C57BL/6 and DBA/2 mice express different Ly alleles, BDF1 cells were susceptible to antisera of both allelic specificities. Treatment of normal 12-day old BDF1 thymocytes with anti-Ly 1.1 or anti-Ly 1.2 antiserum and complement resulted in 60-70% cytotoxicity, as did exposure to anti-Ly 2.1 or anti-Ly 2.2. Fig. 2 shows representative cytotoxicity data from one of four replicate experiments by using the anti-Ly 1.1 or anti-Ly 2.1 reagents. By contrast, between 20 and 40% of thymocytes from virus-infected mice were susceptible to lysis either by anti-Ly 1.1 (or 1.2) or by anti-Ly 2.1 (or 2.2) antiserum (Fig. 2). The reduced sensitivity of virus-resistant thymocytes to anti-Ly 1 or anti-Ly 2 killing suggests that this subpopulation has either fewer cells expressing Ly antigens or that the amount of Ly antigen expression per cell seems to be
Fig. 2. Cytotoxicity with anti-Ly 1.1 and anti-Ly 2.1 sera. Thymus cell suspensions pooled from three to four thymuses of virus-treated and normal BDF; mice were incubated with antiserum and C and cytotoxicity (percent dead cells, stained with trypan blue) was determined. Anti-Ly 1.1 serum (B6 x BALB/c)F1 anti-B6/Ly 1.1 normal thymus, or anti-Ly 2.1 serum, B6/H-2k anti-CE normal thymus, was used with selected rabbit serum (1/10) as a source of C. Antisera were diluted to a point where maximal kill was still observed on normal F1 thymus cell suspensions.

Reduced in the virus-resistant subpopulation. This conclusion was confirmed directly by two indirect immunofluorescence studies, one of which is shown in Table III. Normal thymocytes or virus-resistant thymocytes were exposed to anti-Ly antisera, washed, and subsequently treated with a fluorescein- or rhodamine-conjugated anti-mIg reagent. Such treatment demonstrated that more than 90% of both cell populations could be stained with anti-Ly 1, 2, or 3. Cells were stained with anti-Ly 1 and fluorescein-anti-mIg followed by anti-Ly 2 (or 3) and rhodamine-anti-mIg to determine if both Ly determinants were present on a single cell (as would be inferred from the high percentage of cells positive with either reagent). Such double staining experiments were performed (Table III) and directly confirmed the presence of Ly 1, 2, and 3 determinants (with a somewhat less intense staining then controls) on virus-resistant thymocytes. The Ly phenotype of that population and the suppressor cells contained within it is therefore Ly 1+, Ly 2,3+.

Treatment of the same two cell populations with anti-TL, anti-Thy 1.2, and anti-H-2k antisera and assessment of quantitative cytotoxicity (Figs. 3 and 4) led to the conclusion that the mouse thymic virus-resistant cells were less susceptible to killing with anti-TL and anti-Thy 1.2 sera and more susceptible to killing with anti-H-2k serum than were normal thymocytes. We would infer that the altered susceptibility to these antisera is correlated with an altered expression of the antigens at the single cell level, but such data are consistent also with population shifts in antigen expression. These experiments, taken together, suggest that the suppressor function which we have studied is a property of cells
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**Table III**

*Mouse Thymic Virus-Resistant Thymocytes are Ly 1,2,3+ By Double Immunofluorescence Staining*

| First reagent(s) | Second reagent(s) | Percent positively stained cells and color |
|------------------|-------------------|-------------------------------------------|
|                  |                   | Normal thymocytes | Virus-resistant thymocytes |
| Single staining  |                   |                 |                            |
| anti-Ly 1.2      | fl-anti-mIg*      | 91% ++ (green)   | 90% ++ (green)            |
| anti-Ly 1.1      | fl-anti-mIg       | 95% +++ (green)  | 95% ++ (green)            |
| anti-Ly 2.2      | rh-anti-mIg$      | 90% +++ (red)    | 90% + (red)               |
| anti-Ly 2.1      | rh-anti-mIg       | 90% ++ (red)     | 90% + (red)               |
| anti-Ly 3.2      | fl-anti-mIg       | >95% +++ (green) | >95% ++ (green)           |

Double staining controls

| anti-Ly 1.2 + fl-anti-mIg | rh-anti-mIg | 90% ++ (green only) | 90% + (green only) |
| anti-Ly 1.1 + fl-anti-mIg | rh-anti-mIg | >95% ++ (green only) | 90% + (green only) |
| anti-Ly 3.2 + fl-anti-mIg | rh-anti-mIg | >95% +++ (green only) | >95% ++ (green only) |

* Fluorescein-conjugated goat anti-mouse immunoglobulin; <1% of normal or virus-infected thymocytes stain with this reagent, or with its rhodamine-conjugated counterpart, or after exposure to both reagents.
† Number of ++'s indicates staining intensity.
§ Rhodamine-conjugated goat anti-mouse immunoglobulin.

within a subpopulation of cortical thymocytes with the following surface antigen phenotype: Ly 1+, Ly 2, 3+, TL+, Thy 1+ ↓, H-2+ ↑.

**Size and Distribution of Mouse Thymic Virus-Resistant Suppressor T Cells.** Thymocytes from normal or virus-injected 10- to 13-day old BDF1 mice were analyzed for size and DNA content by using rapid-flow cytofluorimetry. Individual thymic lobes also were prepared for light and electron microscopy. The 5% of thymocytes surviving neonatal infection with mouse thymic virus were characterized by a larger size, greater DNA content by acridine orange staining (Fig. 5), and a localization to the subcapsular outer cortex of the thymus. This subpopulation represents a minor fraction of cells present in the normal thymus and thus seems to have been selected for rather than induced by the cytolytic virus infection. From its size and high DNA content the virus-resistant subpopulation is rapidly dividing with many cells in late S or G2 phases of the cell cycle.

**The Mechanism of Action of Neonatal Suppressor T Cells.** The final set of experiments to be presented here concern the mechanism of the suppression of antibody formation induced by the cells characterized above. These experiments were designed to decide between two alternative modes of suppression; the first would be a reduction in the number of B cells initially triggered by a T-independent antigen (TNP-Ficoll), and the second would be a regulation of B-
Fig. 3. Cytotoxicity with anti-TL and anti-Thy 1.2 sera. Thymus cell suspensions from pooled thymuses of virus-treated or normal BDF1 mice were incubated with antisera and C. Anti-TL serum, \((B6 \times A/TL^{-})F_1\) anti-ASLI (an A strain tumor), or anti-Thy 1.2 serum \((A/Thy 1.1 \times AKR/H-2b)F_1\) anti-ASLI, was used with selected guinea pig serum \((1/4)\) as a source of C. Cytotoxicity with anti-TL on normal DBA/2 thymocytes \(('TL.2')\) is 70% at 1/400.

Fig. 4. Cytotoxicity with anti-H-\(2^b\) and anti-Ly 1.1 + anti-Ly 1.2 sera. Thymus cell suspensions from pooled thymuses were treated with C and serum as indicated in Fig. 2 for anti-Ly sera and as indicated in Fig. 3 for anti-H-2. BALB/c AnN mice were immunized with skin grafts and lymphoid cell suspensions from B6 or \((B6 \times BALB)F_1\) mice to produce a multispecific anti-H-\(2^b\). Anti-Ly 1.1 was mixed with anti-Ly 1.2, C3H/An anti-CE normal thymus, to yield the dilutions indicated on the abscissa. A single incubation with mixed antiserum and C was then performed.
cell differentiation or proliferation. The suppressor cells induced by concanavalin A treatment of adult spleen cells seem to act by the latter mechanism (27) and limit the burst size of each B-cell clone. An experiment designed to determine the number of adult splenic B cells triggered under limiting conditions by TNP-Ficoll in the presence or absence of neonatal suppressor cells is depicted in Fig. 6. This experiment is predicated on the finding that (CBA/N × DBA/2)F1 male spleen cells have an X-linked defect that is limited to their B cells and totally precludes any TNP-Ficoll response (28–30). Such defective F1 male mice have no known T-cell abnormalities including no known difference in suppressor cell function (30). Syngeneic (CBA/N × DBA/2)F1 female spleen cells have normal in vivo and in vitro PFC responses to TNP-Ficoll. Responsive adult F1 female cells were serially diluted into unresponsive F1 male adult spleen cells (lacking neonatal suppressors) or 10-day old neonatal F1 male spleen cells (possessing neonatal suppressor cells) and the mixture stimulated with TNP-Ficoll. The number of cells per culture was constant at 1 × 10⁶. The TNP-specific PFC response per F1 female B cell added to each culture was reduced significantly by dilution into neonatal rather than adult F1 male cells. This result suggests that the suppressive effect of the neonatal spleen cells was due to a reduction of functional B-cell precursor frequency. The alternative possibility, that neonatal cells suppress burst size in a linear dose-dependent fashion, was excluded by the following experiment. Adult BDF1 spleen cells were cultured in the presence or absence of 1 × 10⁶ (10%) mouse thymic virus-resistant spleen cells and the TNP-Ficoll PFC response determined after 40, 48, and 62 h of
Fig. 6. The anti-TNP PFC response (log_{10} converted) of adult normal (CBA/N x DBA/2)F_{1} female spleen cells serially diluted into genetically unresponsive (CBA/N x DBA/2)F_{1} male adult (solid line) or 10-day old (broken line) spleen cells. The antigen was TNP-Ficoll, to which F_{1} male cells are unresponsive because of an X-linked defect in B-cell development. No T-cell abnormalities are known in such mice. Such genetically unresponsive cells therefore are ideal sources of neutral filler cells for cell dilution experiments, as demonstrated by the slope (0.78) of the response per input cell approaching the theoretical limit of one for a single hit phenomenon. Dilution of adult cells into unresponsive neonatal syngeneic cells resulted in a decrease in the number of precursor cells triggered that was proportional to the number of neonatal cells.

culture, before the peak response at 96 h. As shown in Fig. 7, the anti-TNP PFC response is equally suppressed at all time points early in the response. This result is in direct contradistinction to the expected effect of reduction in burst size which would be to show increasing suppression as the duration (and magnitude) of the in vitro response increases. Taken together these two experiments indicate that the main effect of neonatal suppressor T cells is to reduce the number of B cells initially triggered by antigen, and that once triggering has occurred no further suppressive effects are manifested.

Discussion

In these studies we have characterized a subpopulation of lymphoid cells in the neonatal thymus which have been shown to possess marked suppressor activity for antibody synthesis. Such suppressor function is also present in the neonatal spleen and presumably reflects the activity of the same peripheralized
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Fig. 7. The anti-TNP PFC response of adult BDF1 spleen cells cultured in the presence or absence of 12-day old mouse thymic virus-resistant syngeneic spleen cells. The antigen used was TNP-Ficoll and direct PFC were measured at 40, 48, and 62 h of culture. The regression coefficient for the control response is 0.98 and for the suppressed response is 0.87. The slopes of the two lines are identical.

subpopulation of cells, but splenic neonatal suppressor T cells were not analyzed in detail for their surface phenotype because they represent such a small fraction of cells in the spleen (even in neonatally mouse thymic virus-infected mice). The method used to yield a relatively pure, homogenous subpopulation of thymocytes was neonatal infection with mouse thymic virus, an agent previously shown to produce a cytolytic infection of the thymus 7-10 days after infection (15). The 5% of outer cortical thymocytes resistant to the virus were demonstrated to: (a) have greater suppressor activity than cells from intact thymuses, (b) have a Ly 1+, 2,3+, TL+, Thy 1+ ↓, H-2+ ↑ surface alloantigen phenotype; and (c) to be large cells with a greater than average DNA content. From this data we would suggest that the suppressor activity found in neonatal T cells (both in the thymus and probably in the spleen) is a property of large, rapidly dividing cells localized in the outer cortex of the thymus and, by inference, a similar population of cells in the spleen. This inference is based on the absence of T lymphocytes with high Thy 1 expression in the T-dependent areas of splenic lymphoid follicles after neonatal mouse thymic virus infection despite an increase in anti-Thy 1 sensitive suppressor activity. Although we
were unable to directly test the Ly phenotype of the splenic suppressor T cell, we also would infer that it is Ly $1^+$, Ly $2,3^+$ as is the suppressor cell found in the thymus.

A population of thymocytes with similar phenotype, size, and localization has been described (31). On the basis of early incorporation of $[^3H]$thymidine after topical labeling, this cell is assumed to be rapidly dividing and present in the subcapsular region. It has been suggested that such cells are precursors for more mature T lymphocytes, but it would seem inappropriate to call a cell expressing at least two differentiated functions, suppression, and augmentation of a graft-versus-host reaction, immature. If identical cells are found in the spleen, as the presence of suppressor activity would suggest, then such cells must have emigrated from the thymic cortex or have undergone partial differentiation in a nonthymic environment (32). The finding of T cells capable of suppressing B-cell activation in the spleens of nude mice (33) provides some support for the latter concept.

The suppressor T cells found in the thymus and spleen of neonatal mice seem to differ from T cells capable of suppressing immune response in adult animals. Most authors (7-13) have found antigen- or mitogen-induced suppressor cells to express the Ly $1^-$, Ly $2,3^+$ phenotype rather than to be Ly $1^+$, Ly $2,3^+$. Secondly, we have no indication that the administration of antigen or stimulation with concanavalin A (under circumstances which induce suppression in adult spleen cells) has any effect on neonatal suppressor cells (D. E. Mosier and E. Goldings. Unpublished observations). Whether neonatal Ly $1,2,3^+$ cells give rise to adult Ly $2,3^+$ suppressors is unclear.

We have argued previously that neonatal suppressor T cells must directly suppress adult B cells since thymus-independent responses are subject to suppression. This interpretation is strengthened by the experiments depicted in Figs. 6 and 7, which strongly suggest that neonatal suppressor T cells directly react with precursor B cells and prevent antigen-induced differentiation into antibody-forming cells. The data do not exclude, however, an indirect mechanism of suppression mediated, for example, via macrophages or another T-cell subpopulation which has the same phenotype as the T cells which initiate suppression. If B cells are directly subject to suppression, then neonatal suppressor T cells stand in contrast to adult suppressor systems (11) where T cells seems to be the targets of suppressive phenomena. The characterization of the suppressive subpopulation in the thymus suggests one intriguing mechanism for suppression for which we can adduce some very indirect evidence. It has been reported that neonatal thymocytes react by proliferation when confronted with irradiated or mitomycin-treated syngeneic adult spleen cells, generating what has been termed an isogeneic lymphocyte reaction (34). Furthermore, it was shown that the target cells were mature B cells (35). In our experience, this phenomenon is limited to a few strains (notably, CBA) and is quite weak compared to an allogeneic reaction; nevertheless, the possibility exists that cells early in the T-cell lineage (which might be expected to be present in large numbers in the early neonatal period) are not self-tolerant and express autoreactivity directed towards antigens which are predominantly expressed on B cells. The crucial prediction of this model is that at least some members of the T-
cell subpopulation we have studied express self-reactivity which can be confirmed in a system other than suppression of antibody synthesis.

These experiments emphasize one practical point for those studying B-cell function in newborn animals; specifically, the suppressor T cells we have studied express very low amounts of the Thy 1 antigen and therefore are difficult to eliminate by using standard protocols for anti-Thy 1 and complement treatment. Unless depletion of suppressor activity is used as a monitor for abolition of T-cell function, we would suggest that experiments viewing the activity of purified neonatal B cells be interpreted with caution.

Summary

Neonatal suppressor T cells were isolated from the thymuses of 10- to 14-day old BDF1 mice infected at birth with mouse thymic virus. Such cells were enriched for suppressive activity directed against antibody formation by adult B cells and represented a relatively homogenous population of outer cortical cells. Their surface antigen phenotype was found to be: Ly 1*, Ly 2*, TL*, Thy 1* ↑, and H-2* ↓. The cells were larger and contained more DNA than thymocytes from age-matched controls. These findings identify neonatal suppressor T cells as a unique subpopulation separate from most inducible suppressor cells in the adult mouse. The mechanism of action of neonatal suppressor T cells seems to be a reduction in the number of B cells initially triggered by antigen.

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