#72

*Progress in Immunology II, Vol. 2, eds. L. Brent and J. Holborow*




# ALLOTYPE SUPPRESSION AND PRODUCTION(?) BY THYMOCYTES AND THYMUS-DERIVED CELLS*

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During the last few years, immunologists have made considerable progress in defining a number of the functionally interactive lymphocyte populations which collectively make up the immune system. We now recognize and can isolate two broad categories of lymphocytes, T cells and B cells. We know that B cell populations contain the precursors of antibody-forming cells (as well as the AFC themselves) while T cell populations contain the precursors and effector cells operant in cell mediated immunity (CMI). We know also that T cell populations contain at least one other broad functional population which is actively involved in the regulation of the immune responses mounted by T or B cell precursor and effector populations.

The regulator T cell population can be divided according to positive and negative control, and probably further into those cells which regulate T responses and those which regulate B responses. It contains the relatively well defined cooperator (or helper) populations which interact with antigen and B cell[1] or T cell[2] precursors to promote differentiation to their respective effector cells. The regulator population also contains the more elusive suppressor populations which have been shown to prevent appearance of effector cells, i.e. AFC or CMI effectors. Although negative regulation has been difficult to demonstrate, recent studies from a number of laboratories have now confirmed the existence of suppressor cells in a variety of T and B responses. (See review.[3])

Our laboratory has been interested for some time in a population of suppressor T cells which prevents differentiation of normal B cell precursors to antibody-forming cells.[4] These are the cells responsible for chronic allotype suppression in a particular hybrid mouse, SJL x BALB/c. We have pursued these studies in part for their intrinsic interest but also because of their potential value as a model for T cell regulation of B cell function. As things have turned out, we have garnered a fair amount of information about the natural habitat and function of at least one type of suppressor T cell and, in the process, have been led into studies in related areas which have proven quite interesting in their own right.

In this presentation, I will first summarize our work on allotype suppression and then move to a description of collaborative studies in progress in our laboratory on the physical characterization of thymocytes and peripheral T cells. Lastly, together with Melvin and Gayle Bosma, I will present evidence for the rather startling discovery that thymocytes from BALB/c mice either induce or produce donor-type immunoglobulins when transferred to irradiated allotype congenic recipients.

## II. Allotype Suppression**

A few years ago, we showed that perinatal exposure of SJL x BALB/c hybrid mice to antibody to the SJL immunoglobulin allotype (Ig-1b) led to the development of a population of T cells which actively suppressed production of the target allotype. While the extent of suppression was variable in exposed mice, nearly all showed some impairment of the target allotype production and more than half were completely suppressed by 6 months of age, i.e. unable to produce detectable serum Ig-1b levels.

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* This work has been supported by IC-69 and NIH Grants HD 01287 and CA 04681.
** Work on this project has been done by E.B. Jacobson, R. Riblet, C. Metzler, E. Chan.

Studies with spleen, lymph node, thymus or bone marrow from completely suppressed animals showed that all of these tissues contain active suppressor T cells. These cells specifically suppress target allotype production by normal syngeneic splenic B cells when the two cell populations are mixed either in culture[5,6] or in adoptive transfer to irradiated BALB/c recipients. While normal spleen cells cultured or transferred alone were able to produce substantial levels of immunoglobulins or antibodies carrying the allotype, inclusion of suppressor lymphocytes at optimal concentrations reduced allotype production by more than two orders of magnitude, generally to below detectable levels.

The demonstration that the suppressor cells are T cells rests on four kinds of evidence. (See bottom of Table 1.) Taken together, these findings show that a T cell population from suppressed animals is necessary and sufficient for the transfer of suppressor activity.

Table 1.

Summary Description of Chronic Allotype Suppression

Parameters of Chronic Allotype Suppression
- * Occurs in SJL x BALB/c hybrids.
- * Induced by perinatal exposure to anti-allotype serum.
- * Affects $\gamma G_{2a}$ immunoglobulins (allotypes Ig-1a or Ig-1b).
- * Most frequently and completely manifests in animals greater than 6 months of age. Occasional spontaneous reversion observed.
- * Acts in presence of high levels of serum allotype.
- * Transfers to non-irradiated young mice with spleen, thymus, lymph node, or bone marrow from suppressed donors.

Activity of Allotype Suppressor T Cell Population
- * Suppresses allotype production in irradiated BALB/c (600 R) recipients of mixtures of suppressor lymphoid cells and syngeneic normal spleen cells.
- * Suppresses secondary anti-DNP or anti-SRBC antibody production in similar mixture - transfer assay where syngeneic normal spleen cells were antigen-primed.
- * Suppresses anti-SRBC response in vitro (analogous to in vivo mixture-transfer in long term[5] or short term culture.

Evidence that Suppressor Cells are T Cells
- * Thymus is a good source of suppressors.
- * Neonatal thymectomy prevents development of chronic suppression.
- * Suppressors are killed by treatment with anti-Thy-1 (anti $\Theta$).
- * Suppressors are not retained by nylon wool columns which retain B cells but allow T cells to pass.

Recognition that the suppressor is a T cell says a great deal and at the same time says nothing about the mechanism by which suppression occurs. At present, although we have some studies which bear on the question, we are far from a definitive description of how suppressor cells actually prevent allotype production.

One of the approaches we have taken to this problem is an attempt to determine which cell types in the differentiational pathway leading to allotype-producing cells are affected by suppressor cells and whether suppressors kill the affected cells or merely hold them in a non-functional state.

We have shown that spleens from completely suppressed animals are essentially devoid of cells producing the suppressed allotype. Smears of spleen cells from these animals show less than 0.001% of cells whose cytoplasm stains with fluorescent reagents which detect the target allotype (CSC) whereas normal controls show .01% CSC. Partially suppressed animals, as expected, show intermediate numbers of allotype-producing cells.

Since CSC include immature as well as fully differentiated plasma cells, this evidence suggests that suppression interferes with allotype production at least as early as the differentiation of plasma cells from antibody-forming cell precursors (B cells).

Just how early in the pathway the suppressor cells act is more difficult to determine within the framework of current knowledge on the nature of the early and more immediate precursors of IgG producing cells, their mode of expansion, their relationship to immunologic memory and the kinds of surface markers they carry. We can, however, perhaps narrow the range of possibilities a little.

Suppressors clearly do not kill all precursors of allotype producers since suppressed animals retain the potential for production of the target allotype. We regularly observe spontaneous reversion to allotype production for short periods of time in a small percentage of completely suppressed animals. Furthermore, transfer of spleen cells from suppressed animals to irradiated BALB/c recipients with or without the addition of normal syngeneic spleen is almost always followed by a rapid short burst of allotype production before suppression "sets in".

The production of the suppressed allotype in these cases could result from the rapid differentiation of early precursors, however, evidence from partially completed studies suggests that mature precursors exist in suppressed animals. By staining with fluorescent antibody directed at the suppressed allotype we have shown that up to normal numbers of lymphocytes bearing membrane immunoglobulins with the target allotype are present in spleens of suppressed animals even though these same animals have no detectable allotype in circulation and no detectable allotype producing plasma cells in spleen. (See Table 2.) The definitive proof that these cells with membrane-bound allotype are the precursors of allotype producers awaits their separation and functional testing, but by analogy with other systems, it is reasonable to assume that they are indeed precursors.

Table 2

Ig-1b Membrane Staining Cells in Normal and Suppressed Animals

| | PRECURSORS | PRODUCERS | |
|---|---|---|---|
| $(SJL \times BALB/c)F_1$ 8 - 10 months | Ig-1b Membrane Staining Cells (%) | Serum Ig-1b | Ig-1b Cytoplasmic Staining Cells (CSC) |
| Normal Pooled Spleens (2-3 Animals) | 0.49, 0.95, 1.0 | +++ | yes |
| Normal Individual Spleens | 0.74, 1.0 | +++ | n.d. |
| Suppressed Pooled Spleens (2-3 animals) | 0.5, 0.8 | - | no |
| Suppressed Individual Spleens | < 0.008, 0.06, 0.07, 0.1, 0.1, 0.1, 0.24, 0.46, 0.5 | - | n.d. |

The presence of mature precursors in suppressed animals, but the absence of cells further along the differentiational pathway suggests strongly that one way that suppressors prevent allotype synthesis is either by killing diverting or "holding" newly triggered precursors or by preventing the triggering event. This hypothesis is also supported, although not proven, by the demonstration that there is an antagonism between suppressors and cooperators such that suppression of antibody production is reversed either by increasing the cooperators or decreasing the suppressors.

Evidence for this antagonism was obtained in studies of the suppression of production anti-DNP antibodies carrying the target allotype in an adoptive transfer. These experiments are similar to those described earlier in which allotype production was suppressed by mixture and transfer of normal and suppressed spleen.

Irradiated BALB/c animals were given a constant number of DNP-KLH primed normal (non-suppressed) spleen cells as a source of hapten-primed B cell precursors. In addition, they were given both ovalbumin (carrier) primed spleen and unprimed spleen from suppressed animals, each at several concentrations. The challenging antigen was DNP-ovalbumin.

While it was difficult to adjust the cell dosages such that varying degrees of suppression were observed, the results showed clearly that response depended on having more cooperators and fewer suppressors (see Table 3), and therefore that cooperators antagonize suppressors or vice-versa.

Table 3

Antagonism of Suppressor and Cooperator T Cells in Adoptive Secondary Response to DNP

| Suppressor | | Cooperator | | | |
|---|---|---|---|---|---|
| | Suppressed Spleen ($x10^6$) | Ovalbumin Primed 24 | Non-Suppressed 8 | Spleen ($x10^6$) 2.7 | 0 |
| I) | 1.6 | 0 | 0 | n.d. | n.d. |
| | .4 | 50* | 45 | 0 | n.d. |
| | 0 | 330 | 290 | 80 | 0 |
| II) | 3 | 0 | 0 | 0 | n.d. |
| | 1 | 64 | 0 | 0 | n.d. |
| | 0 | 250 | 72 | 23 | 14 |

* Ig-1b PFC/$10^6$ · 4 mice/group, spleens pooled for plaquing.

Lethally irradiated (600 R) BALB/c mice were injected with 12 x $10^6$ hapten-primed (DNP-KLH) spleen cells from non-suppressed (SJL x BALB/c)$F_1$ donors. In addition, each group received various numbers of carrier primed (ovalbumin) spleen cells from non-suppressed (SJL x BALB/c)$F_1$ mice and various numbers of suppressed (SJL x BALB/c)$F_1$ spleen cells. One day after transfer the recipients were challenged with 10 µg aqueous DNP-Ovalbumin and their spleens plaqued on day 7. (Table adopted from C.M. Metzler, et al., (in preparation)).

It is tempting to conclude from this antagonism that suppressors and cooperators compete with each other for a trigger site on the precursor B cell. Occupation of such a site by a suppressor, (or suppressor product), could render the cell unavailable to the cooperator and hence unavailable to the cooperator stimulus necessary to trigger differentiation to an antibody-forming cell. Unfortunately, (or fortunately), however, several other hypotheses which see cooperators and suppressors as acting at totally different points (for example, more cooperators could trigger more precursors, therefore requiring more suppressors to stem

the tide a little further downstream) can be constructed which fit the data equally well. Nonetheless we can conclude from these experiments that the balance between suppressors and cooperators in some way determines the extent of differentiation of cells able to produce anti-DNP antibody carrying the suppressed allotype.

The data on membrane staining presented earlier showing that suppressed animals have putative mature precursors of target-allotype producing cells also suggests a second role for suppressor cells. While some of the suppressed animals tested had roughly the same number of precursors as normal syngeneic controls, others showed many fewer precursors than the controls. It is likely that suppressors then also can reduce the number of precursors, perhaps by preventing lateral expansion or perhaps by preventing differentiation of earlier precursors to mature B cells with membrane bound allotype.

II. Thymocyte and Peripheral T cell Populations[§]

Having shown that the distribution of suppressor T cells was different from other known populations of peripheral T cells (e.g. high activity in bone marrow and thymus), we became interested in characterizing suppressor T cells with an eye to their eventual isolation. This rapidly led to a full blown research project on the characterization of peripheral T cells and thymocyte populations since we clearly had to know from what we were trying to isolate suppressors. The goals of this project fit well with the emerging analysis and separative capacities of the Fluorescence Activated Cell Sorter (FACS) under development in our laboratory with the result that over the past two years, we have made considerable progress in identifying T cell populations according to size, surface antigens, localization, and functional properties.

The conclusions to date from these studies are presented in Tables 4 and 5, which are intended to be self-explanatory. With the aid of the FACS, and in collaboration with investigators from a number of laboratories[2,8], we have identified and characterized three major thymocyte populations and two peripheral T cell populations and determined that isolation of several of these populations by biological methods such as ALS treatment or cortisone treatment yields equivalent populations to those isolated from intact animals by size or surface antigenic properties.

Table 4

Properties of Peripheral T Cell Subpopulations

| Property | $T_1$ (bright Thy-1)* | $T_2$ (dull Thy -1) |
|---|---|---|
| Peripheral lymphoid tissue of highest concentration | spleen | Blood, thoracic duct, lymph node |
| Recirculation | No | Yes |
| Sensitivity to ALS *in vivo* | + | ++++ |
| Removal by thoracic duct drainage | No | Yes |
| Effect of adult thymectomy | In 2 - 6 weeks | After 30 weeks |
| Migration *in vivo* | To spleen > to lymph node | To lymph node > to spleen |
| *In vitro* cytotoxicity | + | ++ |
| TL | - | - |
| Thy-1 (θ) | ++* | + |

*On this scale, thymocytes would be ++++. (Adapted from reference number 2).

§Work on this project (Tables 4 and 5) was done in our laboratory by V. Sato in collaboration with I. Weissman, E. Simpson, H. Cantor, and C.G. Fathman.

Table 5

Properties of Thymocyte Subpopulations

| Cell Size | Large | Small | Medium |
|---|---|---|---|
| % of Thymus | 5 - 7% | 85 - 90% | 5 - 7% |
| Anatomy | Subcapsular zone of cortex | deeper cortex | medulla |
| Life Span | Rapidly turning over | long-lived | long-lived |
| Resistance to cortisone | No | No | Yes |
| Responsiveness to mitogens | No | No | Yes |
| Thy-1 (θ) | +++ | ++ | + |
| TL | ++ | + | - |
| H-2 | + | + | ++ |

There is one significant population absent from the tables as yet: allotype suppressor cells. It is a standard joke around the laboratory that we will get to these soon, but as yet, soon is always next week. Nonetheless, it is likely that in the near future we will be able to identify suppressor cells and perhaps isolate them from spleen, bone marrow, or thymus.

III. Thymocytes as Precursors of Immunoglobulin Producing Cells?*

Dr. Melvin Bosma told us about two intriguing results he and his wife Gayle had uncovered by transferring thymocytes from BALB/c mice to their sublines of an allotype congenic partner strain developed by Dr. M. Potter carrying the b allotype. First, they found that transfer of thymocytes from donors immunized to Ig-1b resulted in long term suppression of production of Ig-1b in the recipients. Second, and perhaps more startling from the standpoint of current dogma, they found that irradiated (450 - 550 R) recipients of relatively few normal BALB/c thymocytes ($3 \times 10^6$ - $10^7$ cells) produced large amounts of immunoglobulin carrying the BALB/c allotype (Ig-1a). With the Bosmas' encouragement, we repeated these experiments in our laboratory with our mouse sublines of M. Potter's strain and found essentially the same results, both with respect to suppression and allotype production. (See M. and G. Bosma, this Symposium.) See also refs. 9 and 10.

We can add to Dr. Bosma's findings that there appears to be a correlation between age of the donor and ability to induce or produce the donor type of immunoglobulin. In several experiments transfers of thymocytes from donors 5 weeks of

*Work on this project was done by T. Tsu and K. Kondo in our laboratory.

age or younger did not lead to donor allotype production in the recipients by 4-5 weeks after transfer, although recipients of cells from donors over 5½ months produced measurable serum levels by two weeks after transfer.

We have also looked at B cell contamination of thymocytes from young and old BALB/c mice as an explanation for this observation. Immunofluorescent staining of thymocyte populations showed that 1) > 99% of the cells were positive with a specific rabbit anti T cell reagent and 2) only 2% or less of the cells were positive for membrane Ig with a reagent which brightly stains 45% of spleen cells. (See Table 6). Since $10^6$ thymocytes are about as effective in transferring allotype production as $10^7$ spleen cells from the same animal, (see Table 7) the contaminating B cells could only be responsible for the allotype production if they were in the order of a thousand times more effective than splenic B cells. While this is possible, it seems more likely that a T cell, i.e. an immunoglobulin negative-bearing and Thy-1 (θ) positive cell is responsible for the immunoglobulin in production.

Whether this T-cell becomes an immunoglobulin producing cell or induces production of a hidden allotype in the recipients, is, as Dr. Bosma stated, still an open question. Our general tendency is to tip in favor of the T cell as precursor. Perhaps as Len (Herzenberg) pointed out earlier, the distinctions usually made between T and B cell functions will turn out to need a little modification. In any event, it is clear that Dr. Bosma's observations have expanded our concept of what T cells can do.

Table 6

Absence of B Cells in BALB/c Thymus

| | | Membrane Ig (B Cells) | | Rabbit α T (T Cells) |
|---|---|---|---|---|
| Tissue | Age | pos/total | % pos | % pos |
| Thymus | 2½ mos | 2/1000 | ∿ 0.2 | all |
| Thymus | 8 mos | 0/1000 | < 0.1 | all |
| | | 1/600 | 0.2 | |
| Spleen | 2 mos | | 42 | 48 |

Table 7

Ig-1a Production in Congenic ($Ig^b$) Recipients of BALB Thymus or Spleen

| Tissue | No. of Cells Transferred | No. of Recipients§ | Ig-1a Serum Level (mg/ml)* Weeks After Transfer | |
|---|---|---|---|---|
| | | | 2 weeks | 3 weeks |
| Thymus | $10^7$ | 4 | > 0.4+ | > 0.3 |
| | $10^6$ | 4 | > 0.3 | > 0.3 |
| Spleen | $10^7$ | 4 | 0.2 | 0.2 |
| | $10^6$ | 4 | 0.04 | 0.04 |
| | $10^5$ | 4 | < 0.03 | < .03 |

*Average Serum Ig-1b level (mg/ml) done by immunodiffusion.
§Recipients were BAB/14 Hz irradiated with 450 R ∿ 18 hrs. prior to transfer. Cells were injected i.v.
+One animal was very low at weeks 2 and 3.

References

1. Transplantation Reviews 1969, Vol. 1 (Munksgaard, Copenhagen).
2. Cantor, H., Simpson, E., Sato, V., Fathman, C.G. and Herzenberg, L.A., 1974, Cell. Immunol. (in press).
3. Gershon, R.K., 1974, in: Contemporary Topics in Immunobiology, Vol. 3, eds. M.D. Cooper and N.L. Warner (Plenum, New York) p. 1.
4. Herzenberg, L.A. and Herzenberg, L.A., 1974, ibid. p. 41.
5. Jacobson, E.B., 1973, Eur. J. Immunol. 3, 619.
6. Herzenberg, L.A., Chan, E.L., Ravithc, M.M., Riblet, R.I. and Herzenberg, L.A., 1973, J. Exper. Med. 137, 1131.
7. Hulett, H.R., Bonner, W.A., Sweet, R.G. and Herzenberg, L.A., 1973, Clin. Chem. 19, 813.
8. Fathman, C.G., Small, M., Herzenberg, L.A. and Weissman, I.L., 1974, Cell. Immunol. (in press).
9. Chou, Chi-tao, Dubiski, S. and Cinader, B., 1966, Nature 211, 34.
10. Chou, C.-T., Dubiski, S. and Cinader, B., 1967, J. Exp. Med., 126, 305.