COMPLETE RESTORATION OF BURSA-DEPENDENT IMMUNE SYSTEM AFTER TRANSPLANTATION OF SEMIALLOGENEIC STEM CELLS INTO IMMUNODEFICIENT CHICKS*

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It has recently been demonstrated that histocompatible cells are required for an effective cellular interaction to occur in antibody formation (1-5), in antigen recognition (6) and in production of germinal centers (5). In our previous study (5) it was observed that immunodeficient chicks cannot be completely reconstituted by transplantation of allogeneic bursal stem cells, even though graft-vs.-host disease was avoided. A full functional and morphological restoration of the bursa-derived (B) immune system was found to require identity of the donor and the recipient at loci near to or identical with those determining the major histocompatibility antigens.

In human medicine, donors sharing with the recipient one HL-A/mixed leukocyte culture (MLC) haplotype are available for cell replacement therapy more often than two haplotype histocompatible donors (7). Therefore, experiments have been carried out to examine whether semiallogeneic bursal stem cells are capable of a complete restoration of the bursa-dependent lymphoid system of immunodeficient recipients. This has been accomplished by using two lines of chickens homozygous at the major histocompatibility locus and their F1 hybrids. The results obtained indicate that semiallogeneic stem cells, in contrast to allogeneic ones, are as effective as histocompatible stem cells in the long-term reconstitution of B-cell functions.

Materials and Methods

Experimental Design.—Chicks treated with cyclophosphamide were transplanted at the age of 3 days with semiallogeneic bursal stem cells (bursa cells from 3-day old donors). The controls included similar transplantations using histocompatible and allogeneic donors, as well as untransplanted cyclophosphamide-treated and normal birds. 4-5 wk after cell transplantation, the chickens were stimulated with sheep red blood cells (SRBC) and Brucella abortus; antibody responses and histological changes in the bursa and spleen were recorded.

Chickens.—White Leghorn line P and line V chickens as well as their F1 hybrids from our own colonies were used. At the major histocompatibility locus, line P is of genotype B2B2 and line V of genotype B13B15. Specific details for the care and housing of the chickens have already been described (8).

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1 Instead of the word "syngeneic," the expression "histocompatible" is preferred here, since our chickens, histocompatible at the major transplantation locus, may show antigenic differences determined by the minor loci.
Cyclophosphamide Treatment.—Cyclophosphamide (Pharmaceutical Manufacturers Lääke Oy, Turku, Finland) was injected intraperitoneally on 4 consecutive days starting on the day of hatching. A dose of 3.0 mg/chick/day was used for the line P chickens and for the F1 hybrids, and 4.0 mg/chick/day for the line V chickens. The difference in the dose is due to a different susceptibility to cyclophosphamide in chickens with different genotypes (5, 9).

Cell Transplantations.—$2 \times 10^7$ bursa cells/chick from 3-day old donors (pooled cells from at least 15 donors on each occasion) were transplanted intravenously into 3-day old cyclophosphamide-treated chicks, more than 6 h after the final cyclophosphamide injection.

Antigen Stimulation.—Antigen stimulation with SRBC and Brucella abortus was accomplished on day 29 and day 36 after the cell transplantation (8). Both antigens were administered on each occasion.

Antibody Titrations, Autopsies, and Microscopic Examination.—Antibody titrations, autopsies, and microscopic examinations were carried out as done previously (8). Blood samples for antibody titrations were collected 7 days after the first stimulation and 5 days after the second stimulation. Autopsies were carried out 5 days after the second antigenic stimulation. Tissue sections were stained with hematoxylin and eosin.

RESULTS

Antibody Formation.—For the long-term restoration of antibody formation to SRBC and Brucella, transplantation of semiallogeneic bursal stem cells was found to be as effective as transplantation of histocompatible bursal stem cells (Table I). Exactly similar results were obtained by transplanting parental cells into F1 hybrids as in the reciprocal donor-recipient combinations. In contrast to these results, transplantation of allogeneic stem cells resulted in a complete restoration of antibody formation only to a thymus-independent antigen, Brucella. After the allogeneic transplantation, anti-SRBC responses were significantly restored ($P < 0.001$ by Student's t-test) only after the first stimulation and only in the other allogeneic combination used, with line V chicks as donors and line P as recipients (Table I).

Microscopic Morphology—In the restoration of bursal morphology of cyclophosphamide-treated chicks, an equally good effect was found after transplantation of semiallogeneic, histocompatible, or allogeneic bursal stem cells. Use of all three types of cells resulted in a morphological reconstitution of the lymphoid follicles of the bursa. The bursal follicles of transplanted birds had a well developed cortex and medulla, and the occurrence of interfollicular connective tissue was normal, as previously described after transplantation of histocompatible bursal stem cells (8). In the untransplanted birds, the follicles appeared rudimentary and devoid of lymphoid cells; the interfollicular connective tissue was increased. Effects on the bursal structure are demonstrated in Table II by the relative weights of the bursa which are known to be reliable indicators of the bursal morphology (8).

Germinal centers are found in the spleen after transplantation of histocompatible bursal stem cells, but not after transplantation of allogeneic bursal stem cells (5). Also in this respect, transplantation of semiallogeneic bursal stem cells resulted in a similar effect as found after transplantation of histocompatible stem cells (Table II). In all parental F1 hybrid combinations used,
### Table I

Antibody Responses after Transplantation of Semiallogeneic, Allogeneic, or Histocompatible Bursal Stem Cells

| Donor line* | Recipients | SRBC§ | Brucella§ |
|-------------|------------|-------|-----------|
|             | Line*      | Cycle-phosphamide | 1st stimulation | 2nd stimulation | 1st stimulation | 2nd stimulation |
| P           | (P × V)F₁ | + | 16 | 3.5 (88) | 6.5 (88) | 1.7 (38) | 4.6 (75) |
| P           | P          | + | 17 | 4.9 (100) | 5.8 (100) | 2.8 (53) | 8.6 (82) |
| P           | V          | + | 16 | (0)  | (0)  | 1.0 (19) | 3.8 (63) |
| V           | (P × V)F₁ | + | 31 | 4.5 (100) | 5.5 (97) | 6.2 (84) | 6.7 (84) |
| V           | V          | + | 13 | 0.7 (38) | 3.7 (100) | 2.6 (69) | 7.2 (92) |
| V           | P          | + | 25 | 2.9 (68) | 0.8 (28) | 4.4 (60) | 6.4 (84) |
| (P × V)F₁   | V          | + | 14 | 0.8 (43) | 3.7 (100) | 2.6 (71) | 5.4 (100) |
| (P × V)F₁   | V          | + | 29 | 3.5 (97) | 4.7 (100) | 5.8 (76) | 7.5 (100) |
| None        | (P × V)F₁ | + | 17 | 0.5 (35) | 0.4 (29) | (0)  | (0)  |
| None        | P          | + | 10 | (0)  | (0)  | (0)  | (0)  |
| None        | V          | + | 13 | (0)  | 0.5 (23) | (0)  | (0)  |
| None        | (P × V)F₁ | – | 13 | 4.2 (85) | 4.8 (85) | 7.8 (100) | 8.2 (92) |
| None        | P          | – | 10 | 3.8 (90) | 5.4 (100) | 6.6 (100) | 9.5 (100) |
| None        | V          | – | 7  | 4.6 (100) | 7.3 (100) | 8.0 (100) | 9.1 (100) |

1st stimulation (both antigens) was given on day 29 after the transplantation and 2nd stimulation on day 36.

* Line P is of genotype B²B²; line V of genotype B¹⁵B¹⁵. 2 × 10⁷ bursa cells from 3-day old normal donors were transplanted into age-matched, cyclophosphamide-treated recipients.

† If decreased during the experiment, the minimum is given.

§ Mean log₂ titers, including non-responders, are given. Figures in parentheses refer to the percentage responding.

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**DISCUSSION**

Our results indicate that semiallogeneic bursal stem cells are capable of a complete restoration of the bursa-dependent immune system of immunodeficient chicks, both in function and in morphology. We interpret these findings to indicate an effective interaction between donor and host cells required for
### TABLE II

**Weight of Bursa Fabricii and Number of Germinal Centers per Cross Section of Spleen 41 Days after Transplantation of Semiallogeneic Bursal Stem Cells**

| Donor line | Recipients | Cyclo-phosphamide | No. | Weight of bursa (mg/100 g body weight) | Germinal centers in spleen |
|------------|------------|-------------------|-----|---------------------------------------|---------------------------|
| P          | (P × V)F₁  | +                 | 15  | 220 ± 25                              | 15.9 ± 4.5                |
| P          | P          | +                 | 16  | 335 ± 19                              | 15.7 ± 3.5                |
| P          | V          | +                 | 16  | 192 ± 18                              | 2.7 ± 1.5                 |
| V          | (P × V)F₁  | +                 | 23  | 411 ± 27                              | 20.4 ± 2.2                |
| V          | V          | +                 | 11  | 254 ± 21                              | 14.7 ± 4.0                |
| V          | P          | +                 | 16  | 273 ± 20                              | 1.0 ± 0.4                 |
| (P × V)F₁ | P          | +                 | 30  | 341 ± 20                              | 21.1 ± 1.5                |
| (P × V)F₁ | V          | +                 | 14  | 223 ± 13                              | 15.8 ± 2.3                |
| (P × V)F₁ | (P × V)F₁ | +                 | 22  | 232 ± 17                              | 21.9 ± 2.5                |
| None       | (P × V)F₁ | +                 | 13  | 55 ± 5                                | 0.0                       |
| None       | P          | +                 | 10  | 69 ± 3                                | 0.1 ± 0.1                 |
| None       | V          | +                 | 12  | 68 ± 3                                | 0.5 ± 0.2                 |
| None       | (P × V)F₁ | −                 | 10  | 437 ± 32                              | 26.3 ± 5.9                |
| None       | P          | −                 | 10  | 471 ± 19                              | 31.6 ± 3.7                |
| None       | V          | −                 | 7   | 489 ± 35                              | 26.7 ± 4.2                |

Mean ± SEM are given.

The antibody formation against a thymus-dependent antigen (SRBC) and for the production of germinal centers. Both of these functions are defective after transplantation of allogeneic bursal stem cells (5). The present findings as such are not applicable to human medicine, since only two lines of chickens with known histocompatibility types were available. They may, however, prove useful for further experimental and clinical studies by demonstrating that the existence of one common major histocompatibility haplotype is sufficient for effective cooperation between cells of host and donor origin. Transplantation of bursa cells provides an ideal model for these kinds of studies, since they are incompetent for graft-vs.-host reactions (5, 10).

We found no difference in the effects of semiallogeneic and histocompatible stem cells. These results are in concert with those of Katz et al. (2–4) who demonstrated excellent cooperative interactions between thymus-derived (T) and B cells originating from parental and F₁ hybrid mice. They employed in vivo cell transfer studies as well as in vitro studies using carrier-primed T lymphocytes and hapten-primed B lymphocytes. There are, however, other studies to indicate that a gene dose effect at the major histocompatibility locus could be involved in the regulation of cellular cooperation of immunocompetent cells. This is suggested by a study done by Kindred and Shreffler (1) on the reconstitution of antibody formation to SRBC by nude mice, as well as by a study done by Rosenthal and Shevach (6) on T-cell macrophage interactions in the antigen recognition in guinea pig. In both of these studies, combi-
nations of semiallogeneic cells allowed a cellular cooperation to an extent which was intermediate to that found to occur in allogeneic and syngeneic cell combinations. Reasons for this discrepancy remain unclear, and it must be recalled that different experimental approaches in different animal species have been used in all the studies referred to.

In addition to the question of a possible gene dose effect at the major histocompatibility locus in cellular cooperation, there is another unsolved problem. Katz et al. consider that histoincompatibility affects effective interaction of T and B cells in the mouse (2-4), and suggest that allogeneic macrophages are as effective as syngeneic macrophages in presenting hapten-carrier conjugates to T and B lymphocytes in the elicitation of secondary antihapten antibody responses in vitro (11). Also, macrophages necessary for a mitotic response in MLC can be either syngeneic or allogeneic to the responding lymphocytes (12–14). On the other hand, data by Rosenthal and Shevach provide evidence that histocompatible guinea pig macrophages are required for antigen presentation in PPD (purified protein derivative of tuberculin)-induced activation of DNA synthesis by immune T cells (6). In the basis of our present and recent data (5) on the inability of allogenic bursal stem cells to induce formation of germinal centers, we are also inclined to believe that in some situations histocompatibility of macrophages and lymphoid cells is essential for an effective cellular cooperation. This assumption is based on the fact that dendritic macrophages and B cells (15, 16) are known to participate in the formation of germinal centers. It is apparent that a solution of these problems, including differences between species, must await further studies with uniform experimental approaches in the mouse, guinea pig, and chicken.

SUMMARY

For transplantation of semiallogeneic bursal stem cells into cyclophosphamide-treated 3-day old chicks, two lines of chickens homozygous at the major histocompatibility locus and their F1 hybrids were used in reciprocal combinations. The semiallogeneic transplantations resulted in a complete restoration of antibody formation to sheep red blood cells (SRBC) and Brucella, of microscopic morphology of bursa fabricii, and of germinal center formation in the spleen. In contrast, allogeneic bursal stem cells were not effective in restoring secondary response to SRBC and germinal center formation, while they were able to reconstitute anti-Brucella responses and bursal morphology. These findings indicate an effective cooperation of donor and host cells leading to a complete restoration of the bursa-dependent lymphoid system, when the donor and recipient share at least one haplotype determining the major histocompatibility antigen complex.

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REFERENCES

1. Kindred, B., and D. C. Shreffler. 1972. H-2 dependence of cooperation between T and B cells in vivo. *J. Immunol.* **109**:940.

2. Katz, D. H., T. Hamaoka, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. II. Failure of physiologic cooperative interactions between T and B lymphocytes from allogeneic donor strains in humoral response to hapten-protein conjugates. *J. Exp. Med.* **137**:1405.

3. Katz, D. H., T. Hamaoka, M. E. Dorf, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. The H-2 gene complex determines successful physiologic lymphocyte interactions. *Proc. Natl. Acad. Sci. U. S. A.* **70**:2624.

4. Katz, D. H., T. Hamaoka, M. E. Dorf, P. H. Maurer, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. IV. Involvement of the immune response (Ir) gene in the control of lymphocyte interactions in responses controlled by the gene. *J. Exp. Med.* **138**:734.

5. Toivanen, P., A. Toivanen, and T. Sorvari. 1974. Incomplete restoration of bursa-dependent immune system after transplantation of allogeneic stem cells into immunodeficient chicks. *Proc. Natl. Acad. Sci. U. S. A.* **71**:in press.

6. Rosenthal, A. S., and E. M. Shevach. 1973. Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. *J. Exp. Med.* **138**:1194.

7. The Copenhagen Study Group of Immunodeficiencies. 1973. Circumvention of early graft-versus-host disease in hemiallogeneic bone marrow transplantation in a case of severe combined immunodeficiency. *Scand. J. Immunol.* **2**:551.

8. Toivanen, P., and A. Toivanen. 1973. Bursal and postbursal stem cells in chicken. Functional characteristics. *Eur. J. Immunol.* **3**:585.

9. Toivanen, P., A. Toivanen, T. J. Linna, and R. A. Good. 1972. Ontogeny of bursal function in chicken. II. Postembryonic stem cell for humoral immunity. *J. Immunol.* **109**:1071.

10. Cain, W. A., M. D. Cooper, and R. A. Good. 1968. Cellular immune competence of spleen, bursa and thymus cells. *Nature (Lond.)* **217**:87.

11. Katz, D. H., and E. R. Unanue. 1973. Critical role of determinant presentation in the induction of specific responses in immunocompetent lymphocytes. *J. Exp. Med.* **137**:967.

12. Alter, B. J., and F. H. Bach. 1970. Lymphocyte reactivity in vitro. I. Cellular reconstitution of purified lymphocyte response. *Cell. Immunol.* **1**:207.

13. Twomey, J. J., O. Sharkey, Jr., J. A. Brown, A. H. Laughter, and P. H. Jordan, Jr. 1970. Cellular requirements for the mitotic response in allogeneic mixed leukocyte cultures. *J. Immunol.* **104**:845.

14. Rode, H. N., and J. Gordon. 1970. The mixed leukocyte culture: a three component system. *J. Immunol.* **104**:1453.

15. White, R. G., V. I. French, and J. M. Stark. 1970. A study of the localisation of a protein antigen in the chicken spleen and its relation to the formation of germinal centers. *J. Med. Microbiol.* **3**:65.

16. White, R. G., and J. Gordon. 1970. Macrophage reception and recognition mechanisms in the chicken spleen. In *Mononuclear Phagocytes*. R. van Furth, editor. Blackwell Scientific Publications Ltd., Oxford, England. 510–527.