

---

## ***Brief Definitive Report***

---

### **CONGENITALLY ATHYMIC NUDE (nu/nu) MICE HAVE Thy-1-BEARING IMMUNOCOMPETENT HELPER T CELLS IN THEIR PERITONEAL CAVITY**

BY HIROMICHI ISHIKAWA AND KAZUHISA SAITO

*From the Department of Microbiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku,  
Tokyo 160, Japan*

A number of *in vitro* as well as *in vivo* investigations established from both functional and histological criteria that congenitally athymic nude (nu/nu) mice are devoid of immunocompetent thymus-derived lymphocytes (T cells) in their secondary lymphoid tissues such as spleen and lymph nodes (LN) (1). Thus, nu/nu spleen cells are not able to mount *in vitro* antibody response to T-dependent antigens such as sheep erythrocytes (SRBC) (2, 3), whereas they can respond well to T-independent antigens like dinitrophenylated polymeric flagellin (3).

In the course of our study, which aimed to characterize the adjuvant effect of nystatin, an antifungal antibiotics, on anti-SRBC antibody response of nu/nu spleen cell cultures (4, 5), we discovered that athymic nu/nu mice have an extraordinary rich source of immunocompetent helper T cells in their peritoneal cavity even if none exist in the secondary lymphoid tissues. In this communication, we describe such basic observations, and discuss the implications of these findings from the current concept of T cell differentiation.

#### **Materials and Methods**

All materials and methods used in this study are essentially the same as those described in our previous papers (4, 5). Briefly, congenitally athymic (nu/nu) mice of BALB/c genetic backgrounds and their euthymic (nu/+) littermates were used at age 8 wk. Uninduced resident peritoneal cells (PC) were collected from the peritoneal cavity of mice by washing with 10–12 ml of sterile ice-cold Hanks' balanced salt solution supplemented with 5% fetal calf serum. For treatment of lymphoid cells with anti-Thy-1.2 antiserum (AKR/J anti-C<sub>3</sub>H/He antiserum),  $5 \times 10^7$  spleen cells or PC were incubated in 1 ml of the antiserum at 1:2 for 20 min on ice. Then, the cells recovered by centrifugation were resuspended in normal guinea pig serum (C') at 1:5 and incubated at 37°C for 20 min. They were washed at least three times before use. To irradiate spleen cells or PC,  $2 \times 10^7$  nucleated cells/1 ml of tissue culture medium was exposed to 2,500 R of X-irradiation (model NELAC 1006, Nihon Denki Co., 1-10 Nitsushim-cho, Fuchu-shi, Tokyo 183, Japan). The components of the complete culture medium, which does not contain 2-mercaptoethanol, as well as the procedures employed for *in vitro* cell cultures were described in a previous paper (5). The cultured cells were harvested, and the number of the direct plaque-forming cells (PFC) in the harvested cells was assayed by the liquid monolayer technique of Cunningham and Szenberg as described (5).

#### **Results and Discussion**

As reported in a previous study (5), spleen cells from nu/nu mice cultured with SRBC did not show any significant anti-SRBC PFC response. Cocultivation of these

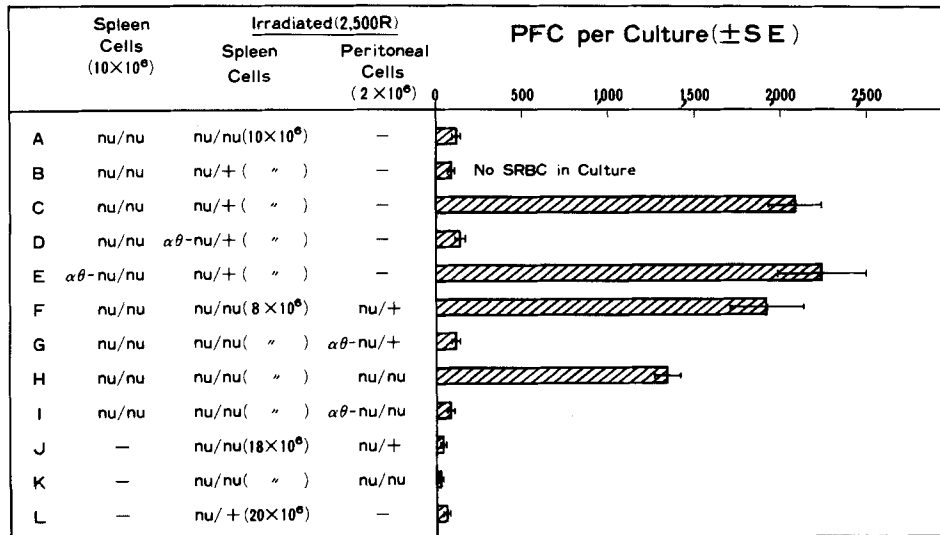


FIG. 1. Effect of cocultivation of irradiated spleen and/or PC on in vitro anti-SRBC response of spleen cells from congenitally athymic nude (nu/nu) mice. The cells used in this experiment were prepared from either nu/nu mice or their littermate nu/+ mice, and the spleen cells and PC were collected from the same animal pool. Absence of thymus was checked in the case of nu/nu mice. A total of  $20 \times 10^6$  cells were cultured according to the method of Marbrook as described (5) and stimulated with  $10 \times 10^6$  SRBC as antigen. Anti-SRBC direct PFC generated in the cultures were determined 4 d after in vitro incubation. The number of anti-SRBC PFC per culture was expressed as a mean  $\pm$  1 SE of the direct PFC generated in triplicate cultures.  $\alpha\theta$ , treated with anti-Thy-1.2 antiserum plus C'.

nu/nu spleen cells with heavily irradiated nu/+ spleen or LN cells resulted in markedly increased generation of PFC in the cultures, whereas no such activities were observed in irradiated nu/nu spleen or LN cells. In one of such reconstitution studies, we examined the restorative effect of irradiated resident PC from nu/+ or nu/nu mice on the impaired anti-SRBC response of nu/nu spleen cells. The results to be noticed were that PC from nu/nu mice restored the response to a similar extent that nu/+ PC did.

The data from a representative experiment from such studies are presented in Fig. 1. The figures in lines A, B, C, and L clearly show that irradiated nu/+ spleen cells are capable of restoring the impaired in vitro anti-SRBC response of nu/nu spleen cells. The treatment of the irradiated nu/+ spleen cells with anti-Thy-1.2 antiserum plus C' completely abrogated the capacity, which indicates that the relevant cell type in the irradiated nu/+ spleen cells is T cell (line D). The same treatment had no effect at all on the activity of antibody-forming cell precursors (B cells) (line E). Similarly,  $2 \times 10^6$  of irradiated nu/+ PC added to the cultures markedly increased the reduced anti-SRBC response of nu/nu spleen cells (line F), and the restoring effect of the irradiated nu/+ PC was sensitive to the treatment with anti-Thy-1.2 antiserum plus C' (line G).

Addition of irradiated nu/nu PC ( $2 \times 10^6$ ) obtained from the same nu/nu donors of the spleen cells restored the in vitro anti-SRBC response of nu/nu spleen cells (line H), although their activity was somewhat less efficient than that of nu/+ counterparts (line F vs. line H). Furthermore, the restorative effect of the irradiated nu/nu PC was

abrogated by treatment with anti-Thy-1.2 antiserum and C', which indicated that the relevant cells in the irradiated nu/nu PC population were T cells (line I). It seems unlikely that the anti-Thy-1 serum treatment affected the macrophage function in PC populations required for in vitro primary antibody response of lymphocytes to SRBC, because the treatment had no effect on the number of adherent cells recovered after 4 d of in vitro incubation (data not shown). Moreover, addition of  $5 \times 10^{-5}$  M 2-mercaptoethanol, one of the potent agents that is capable of substituting the function of macrophages, to nu/nu spleen cell cultures resulted in the augmentation of their decreased anti-SRBC response only to <2.5-fold as in the case of nu/+ spleen cell cultures responding to SRBC. This indicates that the function of macrophages in nu/nu spleen cells is not limited.

These observations would raise important questions: The first is the origin of the Thy-1-bearing immunocompetent helper T cells in the peritoneal cavity of congenitally thymusless nude mice. The second is further characterization of biological significance of these T cells, especially of their ability to perform variety of immunological functions other than helper function on antibody response. In addition, it seems important to assess the actual number of Thy-1-bearing cells in PC populations of nude mice. The fact that we could not detect any significant diminution in the number of viable cells after the treatment of nude PC with anti-Thy-1.2 antiserum plus C' as compared with these after the treatment with C' alone would suggest that the actual number of such cells is quite few.

It is now widely accepted that ~30% of nu/nu spleen cells are committed precursors of T cells or pre-T cells (6, 7) and that these pre-T cells can be induced by either differentiated T cell surface markers or some matured T cell functions after short-term in vitro exposure to various stimuli (7-10). Furthermore, Raff (11) reported the existence of small but significant numbers of Thy-1-positive cells in nu/nu spleens. The origin of these Thy-1-positive spleen cells of nu/nu mice is, at the moment, a question under debate (1, 11).

In this regard, our present observations would raise a possibility that the peritoneal cavity of nu/nu mice can provide a microenvironment where weak but significant extrathymic development of immunocompetent T cells is taking place. Supporting this idea, it would not be fortuitous that macrophages predominate in mouse peritoneal cavity, or that not only thymic factors (9, 10) but also factors derived from macrophages (culture fluid of peritoneal exudate cells) can improve the impaired in vitro anti-SRBC antibody response of nu/nu spleen cells probably by the induction of helper T cells from their precursors (12). Alternatively, in nu/nu mice, small but consistent numbers of immunocompetent T cells are arising somewhere else, and the peritoneal cavity offers a favorable place for accumulation and/or maintenance of such cells. The two alternative possibilities remain to be solved in the future studies. Nevertheless, our results will provide a useful clue to elucidate the possible T cell differentiation pathway in congenitally athymic nude mice.

### Summary

Heavily irradiated peritoneal cells (PC) from congenitally athymic nude (nu/nu) mice markedly restored the impaired in vitro antibody response of nu/nu spleen cells to sheep erythrocyte antigens (T-dependent antigen), whereas irradiated spleen or lymph node cells from nu/nu mice had no effect on the response. This activity of the

irradiated PC of nu/nu mice was completely abolished by treatment with anti-Thy-1.2 antiserum plus normal guinea pig serum (C') and is, therefore, attributable to a function of matured T cells.

The authors would like to thank Dr. Richard W. Dutton for his many valuable discussions and Dr. Takushi Tadakuma for his critical reading of the manuscript. We are also indebted to Tama Yano for her technical assistance and to Ryoza Maeda for his expert taking cares of nu/nu mice.

*Received for publication 28 November 1979 and in revised form 18 January 1980.*

### References

1. Rygaard, J. 1975. Thymus and Self. Immunobiology of the Mouse Mutant Nude. John Wiley & Sons, Inc., New York.
2. Aden, D. P., N. D. Reed, and J. W. Jutila. 1972. Reconstitution of the in vitro immune response of congenitally thymusless (nude) mice. *Proc. Soc. Exp. Biol. Med.* **140**:548.
3. Feldmann, M., H. Wagner, A. Basten, and M. Holmes. 1972. Humoral and cell mediated responses in vitro of spleen cells from mice with thymus aplasia (nude mice). *Aust. J. Exp. Biol. Med. Sci.* **50**:651.
4. Ishikawa, H., H. Narimatsu, and K. Saito. 1975. Adjuvant effect of nystatin on in vitro antibody response of mouse spleen cells to heterologous erythrocytes. *Cell. Immunol.* **17**:300.
5. Ishikawa, H., H. Narimatsu, and K. Saito. 1977. Mechanisms of the adjuvant effect of nystatin on in vitro antibody response of mouse spleen cells: indication of nystatin as a B-cell mitogen and as a stimulant for polyclonal antibody synthesis in B cells. *Microbiol. Immunol.* **21**:137.
6. Roelants, G. E., F. Loor, H. von Boehmer, J. Sprent, L. B. Hägg, K. S. Mayer, and A. Rydin. 1975. Five types of lymphocytes ( $Ig^{-}\theta^{-}$ ,  $Ig^{-}\theta^{+}$ weak,  $Ig^{-}\theta^{+}$ strong,  $Ig^{+}\theta^{-}$ , and  $Ig^{+}\theta^{+}$ ) characterized by double immunofluorescence and electrophoretic mobility. Organ distribution in normal and nude mice. *Eur. J. Immunol.* **5**:127.
7. Sato, V. L., S. W. Waksal, and L. A. Herzenberg. 1976. Identification and separation of pre T-cells from nu/nu mice: differentiation by preculture with thymic reticuloepithelial cells. *Cell. Immunol.* **24**:173.
8. Scheid, M. P., G. Goldstein, and E. A. Boyse. 1975. Differentiation of T cells in nude mice. *Science (Wash. D. C.)* **190**:1211.
9. Scheid, M. P., M. K. Hoffmann, K. Komuro, U. Hämmerling, J. Abbott, E. A. Boyse, G. H. Cohen, J. A. Hooper, R. S. Schulof, and A. L. Goldstein. 1973. Differentiation of T cells induced by preparations from thymus and by nonthymic agents. The determined state of the precursor cell. *J. Exp. Med.* **138**:1027.
10. Blankwater, M. J., L. A. Levert, A. C. W. Swart, and D. W. van Bekkum. 1978. Effect of various thymic and nonthymic factors on in vitro antibody formation by spleen cells from nude mice. *Cell. Immunol.* **35**:242.
11. Raff, M. C. 1973.  $\theta$ -bearing lymphocytes in nude mice. *Nature (Lond.)* **246**:350.
12. Calderon, J., J.-M. Kiely, J. L. Lefko, and E. R. Unanue. 1975. The modulation of lymphocyte functions by molecules secreted by macrophages. I. Description and partial biochemical analysis. *J. Exp. Med.* **142**:151.