PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 146, 30–36 (1974)

# Immunodeficiency and Depletion of Thymic Dependent Lymphocytes in Host Versus Graft Disease (38037)

RICHARD C. HARD, JR.
(Introduced by G. W. Gander)

*Department of Pathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298*

Host Versus Graft (HVG) syndrome is a fatal disease complex that occurs in parental strain mice perinatally inoculated with $F_1$ hybrid spleen cells. C3H mice inoculated neonatally and again at 7 days with $(C3H \times T_6)F_1$ spleen cells developed thrombocytopenia and died of massive intestinal hemorrhage usually within 60 days (1). At autopsy massive lymphosplenomegaly and thymic atrophy were observed. Longer lived $C3H/(C3H \times T_6)F_1$ and $RFM/(T_6 \times RFM)F_1$ mice were found to have renal disease resembling human membranous glomerulonephropathy caused by glomerular deposition of immunoglobulins, presumably as antigen-antibody complexes (2). The supposition that the disease was most likely due to an allogeneic host reaction against donor $F_1$ cells was strongly supported by the finding that the clinical and pathological disturbances could be prevented by neonatal thymectomy or X-irradiation of the host, measures known to suppress immunocompetence (1).

According to the rules of transplantation in inbred mice (3), HVG syndrome ought not have happened. The parental host should either have tolerated or rejected the $F_1$ hybrid cells. $F_1$ hybrid immunocompetent cells have never been shown capable of causing pathological lesions by allogeneic reactions against parental strain hosts. Because of these considerations and because of histopathological changes similar to those of Graft Versus Host (GVH) disease (4), it was postulated that premature exposure of the immature parental strain host to $F_1$ hybrid histocompatibility antigens had led to aberrant immunologic responses (2).

The objectives of the work reported herein were to test the immunocompetency of $RFM/(T_6 \times RFM)F_1$ chimeras in the preclinical stages of HVG disease, and to correlate this result with gross and microscopic tissue alterations. For the initial probe of the immunological status of parent/$F_1$ chimeras before advanced disease had become manifest, antibody response to antigenic challenge with sheep red blood cells (SRBC) was selected. Hemolysin formation, detected as hemolytic plaque forming cells (PFC), has been shown to be a sensitive test of functional, coadjutant components of the immune response system, including thymic derived (T) lymphocytes and bone marrow derived (B) lymphocytes (5) and reticuloendothelial cells (6). To complement this broad spectrum immunological probe, histological studies of the spleens tested for PFC were undertaken, in order to identify significant changes in one or more of these essential cellular components. Previous studies (7) had demonstrated that concentrations of T or B lymphocytes could be identified presumptively by their sites of localization in the spleen. Numerical changes in reticular cells are easily detectable by light microscopy. Data will be presented that implicate depletion of thymic dependent lymphocytes as the cause of the severe immunodeficiency observed.

The mice used were RFM/Al(+) (RFM) and $T_6T_6$ ($T_6$) mice, and their $(T_6 \times RFM)F_1$ hybirds, all from our own conventional colony. The RFM/Al(+)





*Printed in U.S.A.*

subline was derived from outbred RF strain mice, and by now have been inbred for more than 20 generations since their acquisition from Dr. Robert Allen, formerly of Oak Ridge National Laboratory. $T_6T_6$ mice, homozygous for the characteristic chromosome markers, have been similarly inbred since receipt of the Harwell-derived mice from Dr. John Trentin of Baylor University. The $F_1$ hybrids accept $T_6$ and RFM skin grafts indefinitely.

HVG disease was initiated in RFM host mice by neonatal inoculation of ($T_6$ × RFM) $F_1$ hybrid, nucleated spleen cells in dosages of $5 \times 10^6$ intravenously and $10 \times 10^6$ intraperitoneally. A booster dose of $100 \times 10^6$ $F_1$ hybrid spleen cells was given intraperitoneally at 7 days. Control littermates received the same doses of syngeneic RFM cells or were untreated. Because no differences were detected between the controls that had received syngeneic RFM spleen cells (RFM/RFM), and those that had not (RFM), they were considered together. At 21 days, when experimental mice were clinically healthy, but were known from previous work to have marked lymphosplenomegaly, SRBC were inoculated intraperitoneally in doses of $20 \times 10^6$ per gram body weight. Four days later, cell suspensions were made from a portion of the spleen of known weight, quantified and mixed with SRBC, then plated and incubated with guinea pig complement according to the method of Jerne *et al.* (8). The total numbers of splenic cells were calculated. The remainder of the splenic tissue was fixed in buffered formalin for preparation of paraffin sections, cut at the microtome setting of 3 micra, and stained with hematoxylin and eosin, and methyl green pyronin.

At the time of sacrifice, when the mice were 25 days old, both experimental and control groups appeared healthy.

Numbers of splenic cells capable of forming hemolytic antibody following intraperitoneal challenge with SRBC were analyzed as PFC per $10 \times 10^6$ nucleated spleen cells and as PFC per spleen (Table I). By both measures, experimental chimeras were markedly deficient in their responses to SRBC, having on average only 1.3% of control numbers of PFC per $10 \times 10^6$ cells, and only 4.9% of control PFC per spleen. Decreased numbers of PFC per $10 \times 10^6$ nucleated spleen cells in mice with HVG disease might have been interpreted merely as dilution in the enlarged spleens. However, the severe parallel depression in counts of PFC per spleen provided solid evidence of immunodeficiency in the parent/$F_1$ chimeras.

The focus of morphologic studies was on the splenic white pulp. This lymphoid component of the spleen has been separated into two morphologically identifiable components (7), the central periarteriolar area known to be infiltrated with T lymphocytes, and eccentrically situated germinal centers known to be sites of localization of B lymphocytes.

The photomicrograph of the white pulp shown in Fig. 1A was considered representative of normal, 25 day old RFM mice which had received neonatal and 7 day inoculations of syngeneic spleen cells as described above. Moderate numbers of small lymphocytes have accumulated in the periarteriolar area, over a background of reticular cells with indistinct, amphophilic or slightly eosinophilic cytoplasm and large pale-staining nuclei. Inferiorly a hemispherical germinal center has been formed of proliferating reticular cells infiltrated by small lymphocytes.

In contrast, the periarteriolar portions of the white pulp from RFM/($T_6$ × RFM)$F_1$ chimeras (Fig. 1B) was site of a vigorous inflammatory response. The normal complement of small lymphocytes was reduced to negligible numbers (1%). Infiltrating among the reticular cells in their place were polymorphonuclear leucocytes, predominantly neutrophils (66%) but also some eosinophils (15%), together with large mononuclear cells with moderately pyroninophilic cytoplasm (12%), macrophages (3%) and a few plasma cells (3%). Despite the havoc raised in the thymic dependent portion of the white pulp, the adjacent germinal center seemed to have emerged unscathed. The numbers of reticular cells appeared equivalent or even in-

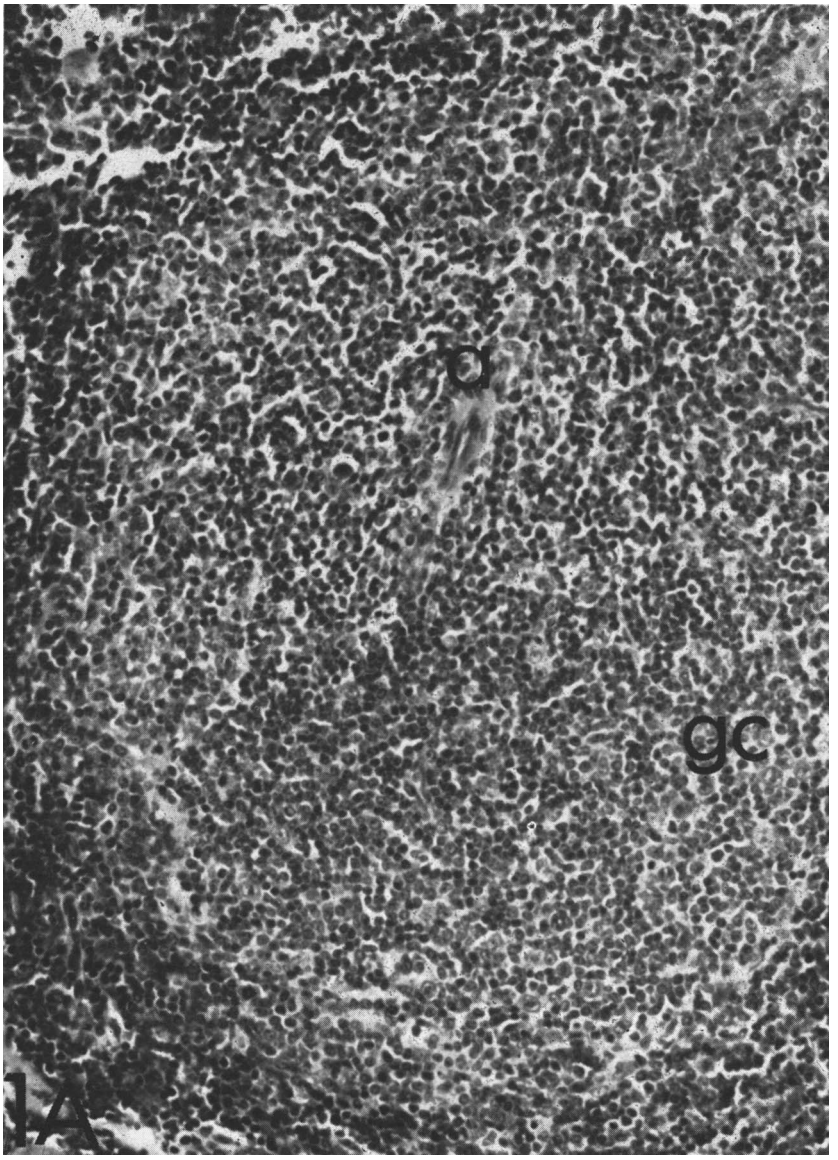


FIG. 1A. This photomicrograph of spleen from a control RFM mouse shows the numbers and types of cells normally found in the white pulp around the central arteriole (a), and the germinal center (gc) at 25 days of age. Typically, 90% of the cells infiltrating the reticular framework of these 2 areas are small lymphocytes. (Hematoxylin and eosin ×190.) 1B. In this section of spleen from a 25 day old RFM/($T_6$ × RFM)$F_1$ chimera there is only a moderate decrease in cellularity around the central arteriole (a), but nearly complete replacement of lymphocytes by neutrophils, eosinophils, large mononuclear cells, macrophages and plasma cells. The large germinal center (gc) has a nearly normal complement of small lymphocytes. Tingible bodies, presumed to be DNA fragments, are characteristically prominent in germinal centers early in HVG disease. (Hematoxylin and eosin ×190.)

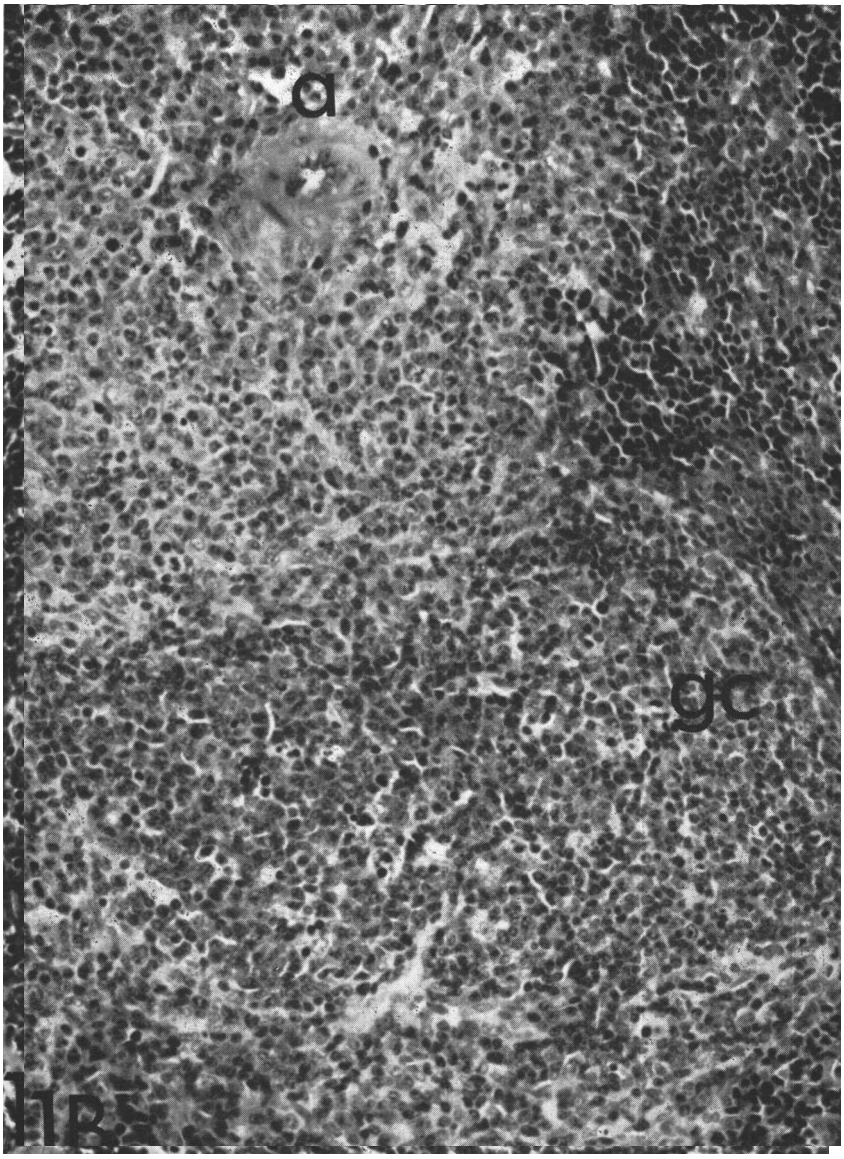


FIG. 1B.

creased when compared with those of the control. Lymphocytes associated with the germinal centers of the experimental mice were present in relatively normal numbers, and acute and chronic inflammatory cells were essentially absent.

When compared with normal organs, the typical thymus from an immunodeficient animal weighed 43% less (Table I). Histopathologic study revealed diffuse and focal depletion of cortical thymocytes. The morphology of all thymi at this stage of HVG disease varied from severe atrophy to normal.

In brief, these immunopathologic investigations have revealed severe immunodeficiency in RFM/($T_6$ × RFM)$F_1$ chimeras coincident with acute and chronic inflammatory changes and lymphocytic depletion in thymic dependent areas of the splenic white pulp. Splenic germinal centers, thought to be sites for antigen trapping and

TABLE I. Thymic and Splenic Weights with Hemolytic Plaque Forming Cells of 25 Day Old RFM/($T_6$ × RFM) $F_1$ Experimental Chimeras Compared with Littermate RFM/RFM and RFM Controls.

| Group | Body weight (g) | Thymic weight (mg) | Thymic index[a] | Splenic weight (mg) | Splenic index[a] | PFC[b] / $10 \times 10^6$ cells | PFC[b] / spleen |
|---|---|---|---|---|---|---|---|
| HVG | 12.7 (7.8–15.7) | 35 (11–63) | 2.7 (1.2–4.7) | 290 (117–514) | 20.4 (15.0–32.8) | 18 (2–69) | 246 (48–604) |
| Control | 12.6 (9.9–14.5) | 61 (53–76) | 4.7 (4.0–5.6) | 73 (45–117) | 5.7 (4.6–8.0) | 1,358 (305–4,113) | 5,004 (2,298–8,314) |

All figures represent mean values, with ranges in parenthesis for 10 experimental and 8 control mice.

[a] Index = $\frac{\text{Organ Weight (mg)}}{\text{Body Weight (mg)}} \times 10^3$.

[b] PFC = Hemolytic Plaque Forming Cells detected 4 days after challenge with sheep red blood cells.

localization of B lymphocytes, appeared to have been spared.

Among several possible interpretations of these results, the most obvious is that the parent strain host has developed an immunodeficiency state due to destruction of T lymphocytes during an unusually vigorous allogeneic reaction against subtolerogenic inocula of $F_1$ hybrid cells. But how to explain this nearly complete devastation of the splenic T cell population?

In light of current knowledge of cell mediated immunity, it could be postulated that an initially specific, allogenic, parent versus $F_1$ reaction caused an inflammatory response (9) in the homing sites for T cells, the great majority of which were destroyed non-specifically as innocent bystanders. With loss of the T cells required as helpers for B cell antibody responses to SRBC, a sharply reduced hemolysin response would be expected. This was exactly the result seen in HVG chimeras, despite apparent preservation of the B cell system. After depletion of host T cells, it could also be anticipated that the aggressive attack on donor $F_1$ T cells would falter, the acute inflammatory response would wane, and rejection of donor erythroid, myeloid and B cells outside the thymic dependent areas might be impaired. Partial confirmation of this hypothetical sequence has been obtained by histopathological observations, and by preliminary studies (10) that revealed persistence of low numbers of donor $F_1$ hybrid cells in the spleens and nodes of old host mice, which could reject $F_1$ skin grafts only after extended periods. Transition from an acute to chronic HVG reaction could, with the help of spared B cells sensitized to donor histocompatibility antigens, lead to formation of antigen–antibody complexes, thought to be the cause of renal disease in parent/$F_1$ chimeras (2).

It should be clear that these morphologic studies at the light microscopic level have not ruled out functional impairment of B lymphocytes as cause of the poor PFC response to SRBC. This important point is under current investigation. Nor have these studies ruled out the possibility that the decreased numbers of PFC were due to

poor antigen handling by a disrupted reticular system (11). In reference to this last possibility, increased activity and efficiency of reticuloendothelial cells have been reported following Graft Versus Host (GVH) allogeneic reactions (12).

The role of infectious agents in HVG disease is problematic at this point. Immunological depression and immune complex nephropathy are among the features of HVG syndrome that have been observed in other animal models but attributed to viral infections (13). Because the lesions of HVG disease may be largely due to an inefficient or inappropriate host response, there are intriguing similarities to the *in utero* infections of rubella and syphilis which are exacerbated by the immunological reaction of the immature host (14). Mouse hepatitis virus—3 has been shown to cause lymphocytic depletion (15), but descriptions of the nature and progression of the lymphoid lesions differed from those of HVG reactions.

Depressed hemolytic PFC responses to SRBC have also been observed in GVH disease (16, 17). In both reports, the investigators attributed inhibition of antibody response in $F_1$/Parent chimeras to antigenic competition. According to their concept, immunocompetent cells were so preoccupied with $F_1$ histocompatibility antigens, they had sharply reduced reserve capacity for response to SRBC antigens. Immunological and morphological similarities to the studies reported here would suggest as an alternative, that the allogeneic GVH reaction had caused sharp reductions in numbers of T lymphocytes, thereby severely limiting any immunological reaction for which they were required. Just as described above for HVG disease, the early stages of GVH disease were also marked by acute and chronic inflammatory reactions and lymphocytic depletion of the thymic dependent, periarteriolar portions of the splenic white pulp (4).

Past and present work has led to the hypothesis that the basic cause of immunodeficiency in HVG, and perhaps GVH, disease is a T cell deficiency induced by allogeneic cellular interactions. It has been difficult to explain satisfactorily the failure of the young parental strain mice to replenish the T cells presumed lost in the HVG battle. Both gross and microscopic observations of the thymus pointed toward organic impairment, but offered no clue to the etiology. It may have been that thymic shrinkage and insufficiency were results of elevated levels of adrenal corticoids, secondary to the stress of the vigorous allogeneic HVG reaction. Such a mechanism has been invoked to explain the thymic atrophy seen in GVH disease (18). Direct attack on $F_1$ hybrid thymus by immunocompetent parental cells has also been suggested as a cause of atrophy in GVH disease (19). Although the possibility of cellular attack does not seem applicable in HVG disease, some other form of $F_1$ versus parent activity has not been excluded. Such an $F_1$ hybrid antiparent immunologic reaction has been reported (20), and recent work has demonstrated that $F_1$ hybrid cells could produce antibody to receptor antigens of parental immunocompetent cells (21). The correct exposition of this allogenic effect on the thymus and thymic derived cells may well provide the key to the mechanisms of fatal HVG and GVH reactions *in vivo*. The similarity of the histopathological findings that follow neonatal thymectomy and GVH (19) and HVG syndromes supports the speculation that a deficiency of T cells may be the common denominator among them.

*Summary.* Host Versus Graft (HVG) syndrome is a fatal disease complex induced in parental strain mice by perinatal inoculations of $F_1$ hybrid spleen cells, and manifested by platelet deficiency, intestinal hemorrhage, massively enlarged lymph nodes and spleen, thymic atrophy and renal disease. In this study, severe depression in splenic hemolytic plaque forming cell responses was observed in 25 day old RFM/($T_6$ × RFM)$F_1$ mice. Histopathological surveys of the spleens revealed that depletion of lymphocytes in the thymic dependent areas of the white pulp accompanied an acute inflammatory reaction. Germinal centers and their accompanying lymphocytes, thought to be of bone marrow origin, were spared. Certain immunological and mor-

phological similarities suggested that Graft Versus Host and Host Versus Graft diseases may have in common an induced deficiency of thymic derived (T) cells.

I thank Fr. G. Brede for plaque assays and Mr. J. Adams, for histological preparations. Supported in part by NIH grant AI-10, 136 and the Louis and Marguerite Privat Memorial Fund.

1. Hard, Jr., R. C., and Kullgren, B., Amer. J. Path. **59**, 203 (1970).

2. Hard, Jr., R. C., Moncure, C. W., and Still, W. J. S., Lab. Invest. **28**, 468 (1973).

3. Snell, G. D., and Stimpfling, J. N., *in* "Biology of the Laboratory Mouse" (E. L. Green, ed.), p. 457, McGraw-Hill, New York (1966).

4. Congdon, C. C., Makinodan, T., Gengozian, N., Shekarchi, I. C., and Urso, I. S., J. Nat. Cancer Inst. **21**, 193 (1958).

5. Claman, H. N., Chaperon, E. A., Triplett, R. F., Proc. Soc. Exp. Biol. Med. **122**, 1167 (1966).

6. Sabet, T., Newlin, C., and Friedman, H., Proc. Soc. Exp. Biol. Med. **128**, 274 (1968).

7. Parrott, D. M. V., and de Sousa, M., Clin. Exp. Immunol. **8**, 663 (1971).

8. Jerne, N. K., Nordin, S. S., and Henry, C., *in* "Cell Bound Antibodies" (D. B. Amos and H. Koprowski, eds.) p. 109, Wistar Institute Press, Philadelphia (1963).

9. Ramseier, H., and Streilein, J. W., Lancet **1**, 622 (1965).

10. Hard, Jr., R. C., Amer. J. Pathol. **70**, 51a (1973).

11. Hanna, Jr., M. G., and Hunter, R. L., Adv. Exp. Med. Biol. **12**, 257 (1971).

12. Cooper, G. N., and Howard, J. G., Brit. J. Exp. Pathol. **42**, 557 (1961).

13. Allison, A. C., Beveridge, W. I. B., Cockburn, W. C., East, J., Goodman, H. C., Koprowski, H., Lambert, P. H., Vanlogham, J. J., Miescher, P. A., Mimms, C. A., Notkins, A. L., Torrigiani, G., Bull. W. H. O. **47**, 257 (1972).

14. Silverstein, A. M., in "Ontogeny of Acquired Immunity," (R. Porter and J. Knight, eds.) p. 17, Associated Scientific Publishers, Amsterdam (1972).

15. Hirano, T., and Reubner, B. H., Lab. Invest. **14**, 488 (1965).

16. Lawrence, Jr., W. L., and Simonsen, M., Transplant. **5**, 1304 (1967).

17. Moller, G., Immunology **20**, 597 (1971).

18. Heim, L. R., Martinez, C., and Good, R. A., Nature **214**, 26 (1967).

19. Miller, J. F. A. P., and Howard, J. G., J. Reticuloendothel. Soc. **1**, 369 (1964).

20. Celada, F., and Welshons, W. J., Proc. Nat. Acad. Sci. **48**, 326 (1962).

21. Ramseier, H., and Lindenmann, J., Transplant. Rev. **10**, 57 (1972).

Received Nov. 8, 1973. P.S.E.B.M., 1974, Vol. 146.