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Hyperimmunoglobulinaemia, T-cell deficiency and plasmacytosis in RFM mice with host versus graft disease induced by the perinatal inoculations of (T6 × RFM)F1 spleen cells

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SUMMARY

Host versus graft (HVG) syndrome may be induced in parental strain mice by perinatal inoculations of F1 hybrid spleen cells. The principal manifestations of the disease include thrombocytopaenia, intravascular fibrin deposits, intestinal haemorrhage, hepatic infarcts, lymphosplenomegaly and renal disease. Immune complexes have been shown to be the cause of the renal lesions, and have been implicated as the triggers for disseminated intravascular coagulation.

In the present studies of RFM mice perinatally inoculated with (T6 × RFM)F1 spleen cells (RFM/(T6 × RFM)F1 mice), quantitative determinations of serum immunoglobulins (Ig) revealed marked elevations of IgG1, IgG2, IgA and IgM. Electrophoretic analyses revealed the polyclonal pattern which typically follows chronic antigenic stimulation. However, IgG1 levels which reached 29 to 72 times control values suggested disruption of homeostatic mechanisms which control circulating Ig levels.

Because antibody responses to histocompatibility antigens were present only occasionally, and then in low titre, it seemed unlikely these antigens were the principal causes of hypergammaglobulinaemia and plasmacytosis.

Morphological studies indicated that the elevated levels of Ig seen in end-stage HVG syndrome correlated well with marked plasmacytosis, the third morphological finding in a sequence that included the precocious development of germinal centres and subsequent depletion of thymic-dependent (T) lymphocytes. The fact that spleen cells from RFM/(T6 × RFM)F1 mice were severely impaired in their capacity to cause graft versus host disease in related (T6 × RFM)F1 and unrelated C3H mice provided strong evidence that the HVG reaction resulted in T-cell depletion, rather than specific immunoincompetence.

INTRODUCTION

Host versus graft (HVG) syndrome has been observed to follow inoculation of F1 hybrid spleen cells into newborn mice of several related parental strains including A (Billingham & Brent, 1958), C3H (Hard & Kullgren, 1970), RFM (Hard, Moncure & Still, 1973), BALB/c and C57Bl/6 (Simpson *et al.*, 1974).

The principal manifestations of the full-blown syndrome are thrombocytopaenia, intestinal haemorrhage, intravascular fibrin deposits, hepatic necrosis, lymphosplenomegaly, thymic atrophy and immune complex nephritis (Hard & Kullgren, 1970; Hard *et al.*, 1973).

Formation of immune complexes appears to be a pathogenetic mechanism of major importance in HVG disease. In addition to causing membranous glomerulonephropathy, the complexes have been implicated as triggers for disseminated intravascular coagulation (Hard & Still, 1975). Of added interest was the finding of organized deposits in some renal lesions, which had previously been described only in patients with systemic lupus erythematosus (Grishman *et al.*, 1967). Prevention of the syndrome by suppression of host immunocompetence provided strong evidence that the direction of the reaction was host against donor (Hard & Kullgren, 1970).

On the basis of transplantation studies in inbred mice (Snell & Stimpfling, 1966), these lethal effects of the F1 cellular inocula on parental strain hosts were originally unexpected. Because they carry the histocompatibility antigens of both parents, F1 hybrid mice are genetically incapable of reacting against either parent. Although more recent work has demonstrated that F1 hybrid cells can form antibodies against receptors on parental cells (Ramseier & Lindenmann, 1972), there have been no reports of pathological sequelae to such reactions. To explain how HVG syndrome could occur, it was postulated that premature exposure of the immune response system to subtolerogenic doses of histoincompatible F1 hybrid cells resulted in a chronic allogenic reaction manifested by the observed lymphoproliferative disorder and antigen–antibody complexes (Hard *et al.*, 1973).

Immunopathological studies done to explore this hypothesis (Hard, 1974a, b) correlated increased immunological reactivity to sheep red blood cells (SRBC) with the precocious appearance of germinal centres and plasma cells in the spleens of very young mice. In slightly older mice, marked depletion of lymphocytes in the thymic-dependent portions of the splenic white pulp and thymic atrophy could be correlated with a severely depressed response to challenge with SRBC.

Morphological changes in lymphoreticular organs, the progressive deposition of immune complexes and the appearance of the nephrotic syndrome all led us to suspect there might be marked alterations in the concentrations of the serum immunoglobulins (Ig). The present studies were undertaken to quantitate serum Ig concentrations and to correlate the observed levels with the progression of changes in the lymphoid organs in moribund RFM mice which had been inoculated perinatally with (T6 × RFM)F1 spleen cells (RFM/(T6 × RFM)F1 mice). Tests for cytotoxic antibodies were done to search for serological evidence of HVG reactivity, and to see if such antibodies might be quantitatively significant components of serum Ig levels. To document further the T-cell deficiency seen in morphological studies, spleen cells from HVG mice were tested for their capacity to cause graft versus host (GVH) disease in related and unrelated recipients.

MATERIALS AND METHODS

Mice. All mice used in these experiments were taken from our own conventional colony. The RFM/A1 (+) (RFM) mice were descendants of out-bred RFM/Un mice (Popp & Amos, 1965), in turn derived from RF mice. They have been maintained by brother–sister matings for more than twenty generations since their acquisition from Dr Robert Allen, formerly of Oak Ridge National Laboratory. Mice of this subline now accept skin grafts permanently from other members of the same subline. Our T6T6(T6) mice, homo-

zygous for the characteristic chromosome markers, have been similarly inbred since receipt of these Harwell-derived mice from Dr John Trentin of Baylor University. The (T6 × RFM)F1 hybrids were always formed by mating T6 females with RFM males. F1 hybrids accept T6 and RFM skin grafts indefinitely. RFM hosts reject (T6 × RFM)F1 hybrid skin between 12 and 16 days after grafting. Our C3H/Bi mice are the inbred descendents of pairs given us originally by the late Dr Carlos Martinez of the University of Minnesota.

Cellular inoculations. All experimental animals were formed by the neonatal inoculation of RFM mice with (T6 × RFM)F1 hybrid spleen cells in dosages of 5×10^6 intravenously and 10×10^6 intraperitoneally. At 7 days, a booster dose of 100×10^6 F1 hybrid spleen cells was given intraperitoneally. Control animals were RFM littermates which had received isogenic RFM spleen cells on the same schedules, or were untreated.

Serum protein studies. All serum studies were done on individual samples of blood drawn from the hearts of mice anaesthetized with sodium pentobarbital. Total serum proteins were determined by the method of Lowry *et al.*, (1951). Electrophoretic separations were done on cellulose acetate strips at pH 8·6 in 0·075 M barbital buffer. Tracings of the Ponceau-S stained strips were made with a Beckman Model R-110 densitometer. Albumin concentrations were calculated from the percentage of the area under the albumin peak and the total serum protein concentration. Immunoglobulin concentrations were determined by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965), using antibody-agar plates and murine Ig standards from Meloy Laboratories.

Cytotoxic antibody studies. Sera from a second series of RFM/T6 × RFM)F1 mice with end-stage HVG disease, and from RFM/RFM and RFM controls were tested for content of cytotoxic antibodies using the technique of Hellström (1959) with some modifications. Thymocytes from (T6 × RFM)F1, RFM and C3H mice were used as targets. Preliminary studies showed (T6 × RFM)F1 cells to be as sensitive targets as T6T6 which were in short supply. Better results were obtained using rabbit complement (Grand Island Biological Co.) diluted 1 : 8 and absorbed with Sea Kem agarose (Cohen & Schlesinger, 1970). Hellström's (1959) index of cytotoxicity was used for comparative purposes: cytotoxic indices = [(percentage viable in standard serum) − (percentage viable in test serum)]/(percentage viable cells in standard serum).

Ninety-four to 99% of the target cells from all three strains were viable after incubation with complement and various titres of standard sera from healthy young adult RFM mice. As a check on the system, the panel of cells were also incubated with sera from RFM mice inoculated with T6T6 spleen cells until a cytotoxic index = 0·50 at a titre of 1/256 was obtained. At titres ranging from 1/1 to 1/16, 94–98% of (T6 × RFM)F1 cells were killed, but cytoxic activity for RFM and C3H cells was essentially 0.

Graft versus host assay. Cell suspensions were made from the spleens of RFM/(T6 × RFM)F1 mice aged 25–63 days of age, and similarly aged RFM/RFM or RFM controls. Nucleated cells from HVG mice were inoculated intravenously and intraperitoneally into five newborn (T6 × RFM)F1 or C3H mice. Littermate recipients received the same dosages of control cells. Because splenomegaly is a prominent sign of both HVG and GVH reactions, death coupled with histopathological evaluations were used to determine if the neonatal inoculations had caused GVH disease. Histopathological criteria included lymphomyeloid depletion of spleen and nodes, reticular cell hyperplasia, thymic atrophy and perivascular infiltrates in the liver (van Bekkum & de Vries, 1967).

Light microscopy. Spleens, lymph nodes, thymi, livers and femurs were fixed in buffered formalin. Sections of all tissues were routinely stained with haematoxylin and eosin (H & E). Spleens, nodes and livers were also stained for cellular RNA content with Methyl Green Pyronin (McManus & Mowry, 1960), modified slightly to compensate for formalin fixation.

RESULTS

Clinical course

Experimental mice appeared healthy and gained weight normally until 5 days or less prior to death. Signs of imminent death included slowed growth or slight loss of weight, lethargy, ruffled fur and decreased body temperature. Facial puffiness and ascites were observed in some cases.

Serum protein studies

The quantitative data on total serum protein, albumin and Ig levels in nine HVG mice

TABLE 1. Quantitative analyses (mg/ml) of serum proteins in RFM/(T6 × RFM)F1 mice in the terminal stages of host versus graft disease and in RFM/RFM and RFM littermate controls

Group	Age (days)	Total protein	Albumin	IgG1	IgG2	IgA	IgM
HVG							
95–8	31	62·7	22·6	15·90	1·70	0·45	0·70
99–4	33	50·3	15·6	13·50	1·55	1·20	0·57
99–7	34	22·5	4·3	5·30	0·40	0·41	0·57
95–5	36	41·3	17·5	9·20	1·01	0·16	0·30
97–4	38	32·0	9·6	8·70	0·92	0·66	0·40
97–7	40	50·8	21·3	10·85	1·35	0·41	0·75
97–10	40	34·6	8·3	5·40	0·48	0·22	0·84
99–6	40	38·2	8·8	8·70	1·01	0·54	0·53
97–5	52	84·0	29·8	16·70	3·20	0·51	0·75
Controls							
95–7	31	47·6	25·3	0·22	0·28	QNS*	0·15
99–5	33	42·8	21·2	0·24	0·44	0·08	0·10
99–3	34	48·8	24·4	0·15	0·39	0·09	0·14
95–6	36	54·0	27·0	0·24	0·36	0·06	0·22
97–3	40	47·2	27·1	0·17	0·51	0·09	0·17
99–1	40	45·5	25·0	0·40	0·77	0·17	0·17
97–2	52	50·3	27·2	0·47	1·16	0·29	0·22

* Quantity not sufficient to perform test.

killed when terminally ill and their seven littermate controls are shown in Table 1. In contrast to the total protein values of controls that vary within the relatively narrow limits of 43–54 mg/ml, total protein levels of HVG mice vary widely, from 23 to 84 mg/ml. Mice with the lowest albumin values had the most severe renal disease and anasarca. Although the serum levels of IgG2, IgA and IgM were nearly always significantly higher than in littermate controls of the same ages, IgG1 levels showed the most spectacular changes. The relative differences between HVG and control mice were probably magnified by the fact that normal mice of the ages studied were in the period of physiological hypogammaglobulinaemia (Fahey & Barth, 1965), when maternal globulins were no longer available and host synthesis was at a slow rate. There appeared to be an irregular increase in the Ig levels of controls with age.

Analyses of the electrophoretic patterns showed that all HVG mice exhibited broad-based elevations of the slow gamma peak (Fig. 1a). This pattern is known to reflect principally high concentrations of IgG, but increased levels of IgA and IgM may contribute to the anodal elevation (Osserman, 1971). In six of the nine cases of HVG, elevated alpha-2 globulin peaks and relative declines in albumin levels (Fig. 1b) heralded the onset of the nephrotic syndrome (Schreiner, 1971). In no case was there a monoclonal spike.

The Ig levels recorded for the control mice appear extremely low, but similar to values published for other strains of this age, using the radial immunodiffusion technique (Fahey & Barth, 1965). The densitometric tracings of control serum protein separations show that the concentrations of proteins migrating in the gamma region are much higher than the

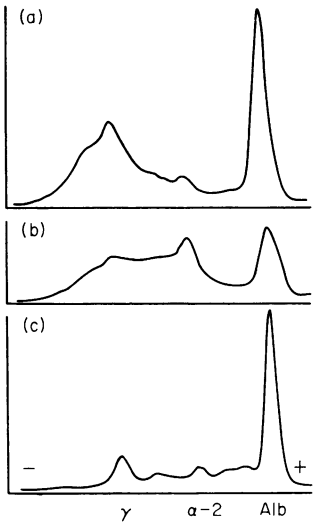

FIG. 1. Densitometric tracings of electrophoretic separations of sera from three mice listed in Table 1. (a) HVG 95–8. The broad-based elevation of the gamma (γ) globulin peak is typical of the pattern seen in cases of lymphoreticular stimulation. (b) HVG 99–6.The elevated alpha-2 (α-2) and low albumin (Alb) peaks indicate that the nephrotic syndrome has been superimposed on the basic condition of lymphoreticular hyperactivity. (c) Control 99–5. The gamma peak is low, as would be expected in the period of physiological hypogammaglobulinaemia.

TABLE 2. Cytotoxic activity of sera from HVG and control mice on related and unrelated thymic cells

RFM/(T6 × RFM)F1 Serum donor	Target thymocytes					
	(T6 × RFM)F1		RFM		C3H	
	Cytotoxic index*	High titre†	Cytotoxic index*	High titre†	Cytotoxic index*	High titre†
228–3	0·05	—	0·07	—	0	—
225–2	0·25	4	0·28	8	Neg	—
234–1	Neg	—	Neg	—	Neg	—
186–4	0	—	0	—	Neg	—
202–1	0·17	8	0·15	4	Neg	—
202–4	Neg	—	Neg	—	Neg	—
176–4	0·79	16	0·66	16	0·03	—
189–12	0·07	—	Neg	—	0·01	—
183–16	0·43	16	0·44	8	0	—
230–3	0·30	4	0·12	4	0·23	4
255–4	0·01	—	0·01	—	0·02	—
Controls‡	0	—	0	—	0	—

* Cytotoxic index $= \frac{\text{(percentage viable, standard)} - \text{(percentage viable, test)}}{\text{(percentage viable, standard)}}$ at dilution of 1 :4.

† High titre = highest titre of test antisera with a cytotoxic index $\geqslant 0{\cdot}15$.

‡ Controls = sera of eight RFM/RFM or RFM mice tested individually.

quantitative Ig studies would suggest. Although usually of lesser magnitude, similar discrepancies are often seen in our clinical laboratory, especially in patients with either hypo- of hyper-globulinaemic conditions.

Cytotoxic antibody studies

It is evident from the data in Table 2 that cytotoxic antibodies were not the major cause of elevated Ig levels in HVG disease. Sera from six of the eleven experimental mice had no detectable cytotoxic activity, and the titres were low in the five which had antibodies. It is of interest that all five mice which had anti-(T6 × RFM)F1 activity also exhibited cytotoxicity for RFM thymocytes in low titre. Lack of reaction against unrelated C3H cells in four of five cases suggested specificity of the reaction.

Graft versus host reactivity

Spleen cells from mice with HVG disease were unable to cause GVH disease in related (T6 × RFM)F1 mice (Table 3). Their capacity to cause GVH disease in unrelated C3H mice was also shown to be severely impaired. C3H and F1 survivors of the RFM/(T6 × RFM)F1 inocula were killed after observation periods ranging from 64 to 222 days. All were found free of any histopathological lesions that could be associated with GVH

TABLE 3. Capacity of spleen cells from RFM/(T6 × RFM)F1 mice with HVG syndrome to cause graft versus host disease in (T6 × RFM)F1 and C3H mice

Donor	Spleen cells ($\times 10^6$) i.v.*	i.p.*	Newborn host	Survivors†
HVG (6)‡	10		(T6 × RFM)F1	21/21
RFM (5)	10		(T6 × RFM)F1	4/18
HVG (5)	5–10	9–10	(T6 × RFM)F1	15/15
RFM (5)	8–11	8–11	(T6 × RFM)F1	0/15
HVG (10)	10	10	C3H	24/29
RFM (12)	10	10	C3H	1/21

* i.v. = Intravenously; i.p. = intraperitoneally.

† Survivors = number of mice alive 60 days or more after neonatal inoculation of donor spleen cells, divided by total number of recipients.

‡ Numbers of donor mice.

disease. Five of the twenty-nine C3H mice were dead 15–48 days after their neonatal inoculation with HVG cells. Two died with the stigmata of GVH disease, one died with a myocardial abscess, one died with lobar pneumonia, and the cause of death was undetermined in the fifth. Of the fifty-four F1 and C3H recipients of RFM cells, forty-six were dead by 21 days, with runting, diarrhoea, and lymphoid depletion of thymus, nodes and spleen. Four more were dead by 64 days with shrunken spleens, nodes and thymi. The four long-term survivors were healthy when killed at 65–187 days of age.

Histopathology of lymphoreticular and myeloid organs of HVG mice

The morphological changes seen in the spleens and lymph nodes of RFM/(T6 × RFM)F1 mice occurred in a sequential pattern that varied remarkably little in its timing and intensity from one animal to the next. Reticular cell hyperplasia and the premature appearance of germinal centres and plasma cells were the earliest detectable changes. Next there was a heavy infiltration of polymorphonuclear leucocytes into the thymic-dependent areas of spleens and nodes which always spared the germinal centres. Severe depletion of lymphocytes in thymic-dependent areas followed. The principal differences between HVG spleens and lymph nodes included a delay of about 5 days in the sequence of histopathological alterations, and excessive numbers of plasma cells in the sub-cortical and medullary zones of the lymph nodes (Fig. 2). Plasmacytosis was also prevalent in the numerous perivascular infiltrates of the liver (Hard & Still, 1975). Plasma cells were seen occasionally in films of peripheral blood, but never in thymus, and rarely in bone marrow.

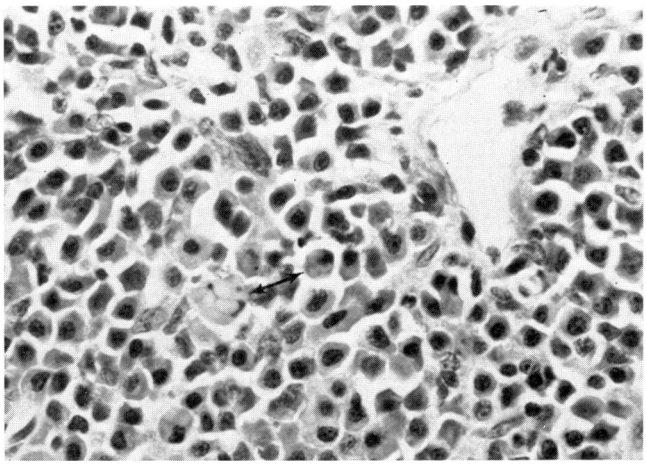

FIG. 2. Lymph node from a 40-day old RFM/(T6 × RFM)F1 mouse. The medullary portion seen here is composed almost exclusively of plasma cells. Two Russell body cells (arrow) are also present. These cells are commonly seen in hypergammaglobulinaemic conditions (H and E; magnification ×480.)

DISCUSSION

These studies of mice with HVG syndrome have established that polyclonal gammopathy follows a predictable sequence of morphological changes in the lymphoreticular organs, which includes the precocious appearance of germinal centres, T-cell depletion and hyperplasia of plasma cells. The high concentrations of IgG1 with lesser increases in IgG2, IgA and IgM are typical of the pattern of Ig elevations seen after chronic antigenic stimulation (Talal *et al.*, 1964; Coe, 1966), allowing for variations in antibody responses among inbred strains of mice (Fink & Quinn, 1953; Barth, McLaughlin & Fahey, 1965). The heterogeneity of the Ig response was further documented by the electrophoretic studies which revealed broad-based globulin peaks and lack of a monoclonal spike. Clinically, this pattern of diffuse

hypergammaglobulinaemia is associated with conditions causing generalized hyperactivity of the reticuloendothelial system such as chronic infections and collagen diseases, especially systemic lupus erythematosus (Osserman, 1971). The hyperglobulinaemia of late HVG disease correlated very well with the hyperplasia of plasma cells. It has been shown that elevated levels of gamma-globulins are due to increased production by plasma cells rather than decreased catabolism (Fahey & Robinson, 1963).

However, levels of IgG1 that reached twenty-nine to seventy-two times control values and the progressive deposition of immune complexes indicated responses which were something more than the normal reactions to chronic antigenic stimulation. Such marked deviations would suggest disruption of the homeostatic mechanisms regulating serum Ig levels in general (Fahey & Robinson, 1963), and perhaps disruption of feedback mechanisms regulating specific antibody formation in particular (Rowley & Fitch, 1964).

It seemed logical to expect that foreign antigens present in the donor (T6 × RFM)F1 cells might be the major source of stimulation of plasmacytosis and hyperimmunoglobulinaemia. However, serological evidence of HVG reactivity has been variable. Although cytoxic antibodies and positive Coombs' tests (Hard & Kullgren, 1970) have been observed in occasional HVG mice, the irregularity of such findings makes it unlikely that either histocompatibility or red cell antigens were the principal stimuli for overproduction of Ig. It is equally unlikely that they were the main antigenic components of the immune complexes deposited in the kidneys of HVG mice. Some sera from RFM/(T6 × RFM)F1 mice appeared to have an antibody cytotoxic for isogenic RFM thymocytes that may be similar to the autoantibody observed by Boyse *et al.* (1970) following immunizations with allogenic cells. Its low titre and variable presence make its importance difficult to assess. Thus, the identities of the antigenic stimuli presumed responsible for elevated serum Ig levels and formation of antigen–antibody complexes in HVG disease remain unknown.

It is generally accepted that GVH reactivity is one of the functions of T cells (Raff, 1973). The finding that spleen cells from older HVG mice lacked the capacity for GVH reactivity against related (T6 × RMF)F1 mice thus helped to corroborate earlier morphological observations of severe lymphocytic depletion in thymic-dependent areas of the spleen (Hard, 1974a, b). The demonstration of markedly impaired GVH reactivity against unrelated C3H strain mice extends previous observations (Hard, 1974c; Simpson *et al.*, 1974), and provides even stronger evidence that the HVG reaction results in T-cell deletion rather than specific incompetence. The mechanisms are unknown which cause loss of both donor F1 and host T lymphocytes. The influx of large numbers of polymorphonuclear leucocytes into thymic-dependent areas and premature thymic atrophy are undoubtedly of pathogenetic significance.

It seems difficult to attempt any explanation of the observed hyperimmunoglobulinaemia and plasmacytosis without taking into account the T-cell loss, which is morphologically apparent as early as 10 days, and progresses to nearly total depletion by 25 days in RFM/(T6 × RFM)F1 mice (Hard, 1974a, b). There are indications that some immunoregulatory mechanisms involve distinct subpopulations of T cells which activate (Claman, Chaperon & Triplett, 1966) or suppress (Herzenberg *et al.*, 1973) the antibody-forming B-cell system, of which plasma cells are a part.

In this light it is instructive to compare the HVG model with other murine models of T-cell depletion. Table 4 was constructed to help compare the impact of T-cell loss at various ages on serum Ig levels. From the information in Table 4 and other sources two points can be inferred. (1) T cells seem essential as starters (helpers?) for production of classes IgG1,

TABLE 4. Relative Ig concentrations in experimental mice depleted of T cells at varying ages

Group	Time of T-cell loss	IgG1	IgG2	IgA	IgM
Nude	Prenatal	−2	−2	−3	0
Newborn thymectomy	Perinatal	−2	−2	+1	0
HVG	Prepubertal	+3	+2	+2	+2
NZB	Adult	0	+1	+1	+3

Numerical values represent the degree of change in serum immunoglobulin levels of experimental mice, relative to values observed in control mice assigned the value of 0.

Range = −3 (very low concentrations) to +3 (very high concentrations). For sources, see text.

IgG2 and IgA, but not IgM. This is the main implication of the quantitative Ig studies of Bloemmen & Eyssen (1973) in nude mice, which suffer from thymic agenesis and nearly complete lack of thymic-derived cells. (2) If even small numbers of T cells escape the thymus and activate portions of the B-cell system, later depletion may result in proliferation of plasma cells and hyperimmunoglobulinaemia.

This seems a reasonable interpretation of the observations that elevated levels of one or more Ig and reticular and plasma cellular hyperplasia are findings common to mice depleted of T cells after birth because of neonatal thymectomy (Fahey, Barth & Law, 1965; Parrott & East, 1962), HVG reactions (*vide ante*) or unknown causes in the case of the New Zealand Black (NZB) strain (Warner & Wistar, 1968; East, de Sousa & Parrott, 1965). At first glance it would seem that the low levels of IgG1 and IgG2 observed in neonatally thymectomized mice did not correlate with the plasmacytosis, and thereby argued against the basic premise. However, it has been shown that the production of Ig was normal or slightly increased in these animals, but the rate of catabolism was greatly increased (Fahey *et al.*, 1965).

Consideration of these points has led us to develop a working hypothesis to explain the plasmacytosis and hyperglobulinaemia of HVG syndrome. We have postulated that the severe and non-selective loss of T lymphocytes include helper T cells, and T cells (suppressors?) involved in the feedback control of Ig synthesis. Loss of helper T cells would explain the impaired responses to thymic-dependent antigens (Hard, 1974a, b). Loss of putative 'controller' T cells after partial activation of the B-cell system could result in nearly autonomous proliferation of plasma cells synthesizing without restraint, Ig with a range of reactivity which would be limited by the range of antigenic experiences gained when helpers were present. This scheme also has the merit of providing an explanation of poor primary antibody responses in the face of high levels of immunoglobulins.

The relative inability of HVG mice to raise antibodies to donor cellular antigens has raised the suspicion that an infectious agent might be the stimulus for hypergammaglobulinaemia, immune complex formation and plasmacytosis. A possible mechanism is suggested by the finding of non-neutralizing antibodies to the Aleutian disease virus which cause a similar triad in mink (Porter & Larsen, 1967). Because the antibodies are

apparently ineffective in curtailing viral replication, viral antigens would persist and continue to stimulate responsive cells, Ig synthesis and immune complex formation. The source of chronic antigenic stimulation in HVG disease could be a murine leukaemia virus (Upton *et al.*, 1966), against which many strains have been found capable of raising antibodies (Nowinski & Kaehler, 1974). It is well known that chronic allogenic GVH reactions can activate endogenous oncorna viruses (Hirsch *et al.*, 1970). Simpson *et al.* (1974) were able to isolate murine leukaemia virus from two out of three mice with long-term HVG disease. Loss of homeostatic control over Ig formation by T cells coupled with chronic antigenic stimulation by activated endogenous viruses could lead to the relentlessly progressive formation of immune complexes seen in HVG disease. Fudenberg (1971) has postulated such a mechanism to explain the aetiology of some autoimmune diseases and lymphoid neoplasms seen clinically. We have suggested (Hard *et al.*, 1973) that premature exposure of the immune response system to maternal cells might predispose human infants to a similar sequence of events.

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