Brief Definitive Reports

INFECTIOUS AGAMMAGLOBULINEMIA: TRANSMISSION OF IMMUNODEFICIENCY WITH GRAFTS OF AGAMMAGLOBULINEMIC CELLS

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The chicken provides a unique model to study mechanisms of humoral immune responsiveness since agammaglobulinemia can be readily induced in birds by a variety of procedures which result in removal or ablation of the bursa of Fabricius and post bursal peripheral lymphoid cells during embryonic or early extraembryonic life. During the course of experiments utilizing adoptive cell transfer to study the effect of cells from immune agammaglobulinemic chickens on the subsequent immune response of normal birds, it was unexpectedly found that adult normal chickens receiving bone marrow cells from 4-mo old agammaglobulinemic donors subsequently developed progressive humoral immunodeficiency and agammaglobulinemia.

Materials and Methods

Newly hatched line 6, subline 1 chicks,[1] homozygous at the major histocompatibility locus (B_7B_7) were surgically bursectomized 1 day after hatching. Normal and bursectomized chicks were then given 550 R whole body X-irradiation. Following this treatment approximately 85% of the bursectomized birds are agammaglobulinemic at 10 wk of age as determined by loss of detectable serum immunoglobulin (less than $1/1000$ of the adult normal IgG level). In addition their spleens lack B lymphocytes detectable by either indirect immunofluorescence with rabbit antichicken L chain or antichicken γ- and μ-chain antisera (1). Irradiated unoperated birds develop normal immunoglobulin levels and have normal numbers of B lymphocytes.

Serum immunoglobulin levels were determined by radial diffusion in agarose-containing rabbit antisera specific for chicken γ- or μ-immunoglobulin heavy chains. Results are expressed as a percentage of the value obtained with a standard pool of adult chicken serum. Lower limits of detection are 1% for IgM and 0.1% for IgG. Serum antibody to KLH was determined in microtiter by passive hemagglutination of KLH-coated chicken erythrocytes using chromic chloride (2) as the coupling agent.

Bone marrow was obtained from previously heparinized donor birds (500 μ) by flushing the long bones with balanced salt solution. A single cell suspension was prepared by consecutively drawing the bone marrow into syringes fitted with 16, 19, 22, and 25 gauge needles.

[1] Chickens were obtained from Dr. Howard Stone at the USDA Regional Poultry Research Laboratory, East Lansing, Mich. This strain of chicken was bred in closed families from 1939 until 1962 when strict brother-sister inbreeding procedures were instituted which are now in their 12th generation. When extensively studied in 1969, these birds were homozygous for the B_7 allele at the major histocompatibility locus as well as homozygous at RBC alleles A_4, E_7, C_5, D_3, H_2, I_2, L_1, and P_4.

Results

When the bursectomized-irradiated agammaglobulinemic and control birds were 4 mo of age, a group of control birds were given 350 R whole body X ray on 3 consecutive days. Following the last dose of irradiation one group of six irradiated birds was given an intravenous injection of 3.7×10^9 pooled bone marrow cells obtained from five agammaglobulinemic donors; a second group of 10 irradiated birds was given an equal number of bone marrow cells pooled from eight normal donors; and a third group of eight irradiated birds received no cell transfer. 6 days later, each bird was given an intramuscular injection of 10 mg keyhole limpet hemocyanin (KLH) emulsified in Freund's complete adjuvant.

Fig. 1 shows the titer of anti-KLH antibody in the serum of the three groups as determined by passive hemagglutination of KLH-coated chicken erythrocytes. The recipient chickens used in this experiment had an average preimmunization titer to KLH of 1/64, presumably reflecting natural antibody to some cross-react-

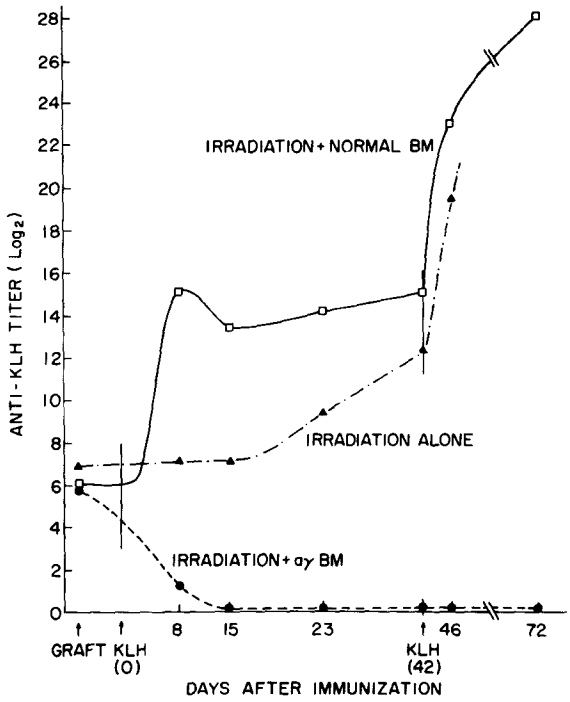

FIG. 1. Anti-KLH antibody titer in the three experimental groups following immunization at day 0 and reimmunization at day 42.

ing environmental antigen. Those irradiated birds which did not receive adoptive cell transfer showed no change in titer for 2 wk after immunization, but by 6 wk had increased titers averaging five serial twofold dilutions. During this 6-wk period, two of the eight birds in this group died, probably as a consequence of the irradiation (approximately LD_{25} – ?).

The anti-KLH response of the irradiated chickens grafted with normal bone

marrow was prompt and quite striking. By marked contrast, those birds receiving an adoptive transfer of bone marrow cells from agammaglobulinemic birds showed no such antibody response. In fact by 21 days after cell transfer (15 days after immunization) their sera had completely lost all anti-KLH antibody activity. After secondary challenge with KLH 42 days after primary immunization, both irradiated control birds and irradiated birds receiving normal bone marrow responded with a prompt antibody rise. However, again the recipients of the agammaglobulinemic cells failed to produce detectable antibody.

In view of this striking failure of antibody responsiveness in the birds receiving agammaglobulinemic bone marrow, serum immunoglobulin levels in each group of birds were measured, Table I. By 21 days following adoptive cell transfer, IgM was no longer detectable in the sera of birds grafted with agammaglobulinemic cells while IgG had fallen to 30% of the pretransplant level. By contrast, the sera of the two control groups had 63 and 68% of their initial IgM and 129 and 132% of their initial IgG. By 55 days following adoptive cell transfer, IgG was no longer detectable in the sera of any bird grafted with agammaglobulinemic cells. Follow up examination of these birds up to 9 mo after cell transfer demonstrated persistent failure of specific antibody responsiveness and agammaglobulinemia.

TABLE I

Serum Immunoglobulin Levels (Group Mean)*

Treatment group	IGM		IgG		
Days after grafting:	0	21	0	21	55
Irradiation alone	241	165	109	144	
Irradiation + normal cells	127	81	62	80	
Irradiation + aγ cells	116	<1	44	13	<0.1

* Expressed as a percentage of the value obtained with a standard serum pool prepared by mixing serum from 10 adult chickens.

Discussion

The development of agammaglobulinemia in sublethally irradiated normal chickens given bone marrow from agammaglobulinemic donors is striking and unexpected. As the control group demonstrates the amount of irradiation used in these experiments is insufficient in itself to cause such profound humoral immunodeficiency. In fact, in preliminary experiments we have found that frequently agammaglobulinemia is transmissible with only a single 350 R pretreatment of the recipient, and that occasionally agammaglobulinemia develops in recipients of agammaglobulinemic cells without any prior irradiation of the recipient.

The possibility that the transferred agammaglobulinemic cells are simply displacing the recipients' own B-lymphocyte population because of an advantage provided over these host cells by irradiation is conceivable but it seems quite unlikely that such displacement would be total and that at least a few host B

cells would not survive to respond at a later time. Utilizing a similar transplantation protocol between normal birds of different sexes, a state of chimerism with cells of both donor and host origin in spleen and bone marrow has been found for at least 4 mo following transplantation (R. M. Blaese and J. Whang Peng, unpublished observations).

Graft-vs.-host (GVH) reactions can result in humoral immunodeficiency (3). Although this strain of chicken has not undergone strict inbreeding long enough to be considered completely syngeneic, it is homozygous at the major histocompatibility locus and cells from birds of this strain do not stimulate each other in mixed lymphocyte culture (R. M. Blaese and H. Kirchner, unpublished observations). In addition, we have never observed anything which appears to represent GVH in our transplanted birds and furthermore, if GVH were responsible for the humoral immunodeficiency, one might expect to have observed a similar finding in recipients of normal marrow.

In the neonatally thymectomized or adult thymectomized-irradiated mouse, stem cells present in the bone marrow are capable of differentiating into competent T lymphocytes if the thymus-deprived animal is provided with a thymic graft (4–6). Similarly one might expect that precursor stem cells in the bone marrow of the agammaglobulinemic birds might be capable of differentiating into competent B lymphocytes under the influence of the bursa of Fabricius of their new host (7–9). If the normal B-lymphocyte population in the recipient chickens is deleted by the X ray and bone marrow grafting, then the development of agammaglobulinemia in these chickens suggests that either such precursor stem cells are no longer present in the bone marrow of 4-mo old agammaglobulinemic chickens or that the recently irradiated, moderately involuted host bursa cannot function to cause their differentiation into B cells. However, since we have shown that irradiated control birds do not develop immunodeficiency and that a state of chimerism exists in the bone marrow and spleen of similarly irradiated birds receiving grafts of normal bone marrow, the possibility that the grafted agammaglobulinemic cells are exerting some form of active suppression of humoral immunoresponsiveness seems a more likely alternative.

A number of studies in rodents have demonstrated active immunoregulatory cells capable of profoundly altering humoral immune responses. Baker, et al. demonstrated a T-lymphocyte regulatory influence on the antibody response of mice to pneumococcal polysaccharide (10). Gershon and Kondo (11) have described the requirement for thymus-derived cells for the induction and transmission of "infectious immunological tolerance." Even more closely related to our observations are those of Jacobson, Herzenberg, and associates (12–15). They have demonstrated that the phenomenon of specific immunoglobulin allotype suppression can be induced in normal cells by the transplantation of irradiated "indicator" mice with cells obtained from nonsuppressed donors mixed with θ-positive thymus-derived lymphocytes from allotype suppressed donors. It is indeed tempting to speculate that the adoptive transfer of agammaglobulinemia observed in the chicken model may similarly reflect such an active lymphocyte mediated suppression and also that certain humoral immunodeficiency states in man may be related to an active immunosuppressive mechanism rather than simply a deficiency in certain B lymphocytes or their precursors.

Summary

Agammaglobulinemia induced in chickens by surgical bursectomy and irradiation at hatching is transmissible at 4 mo of age to sublethally irradiated normal chickens by bone marrow transplantation. The immunodeficiency which develops in the recipients persists for life and is characterized by inability to produce antibody and by progressive loss of detectable serum IgM and IgG.

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