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Restricted Expression of an MHC Alloantigen in Cells of the Erythroid Series: A Specific Marker for Erythroid Differentiation

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The spectrum of reactivity with various types of cells of a monoclonal antibody (CH-4) which detects a private MHC antigen of chickens was analysed. CH-4 agglutinates only RBCs that possess the B^2 (MHC) haplotype. A new rosette-forming cell (RFC) assay was devised to detect individual cells (excluding RBCs) that possess the CH-4 specificity on their cell surfaces. RBCs that have CH-4 chemically coupled to their surfaces attach to, and form rosettes with, B^2 antigen-bearing cells. Most non-RBC RFC were detected in active erythropoietic organs (adult bone marrow and embryonic spleen), and none were found in organs where erythropoiesis does not occur: adult thymus and bursa. Preincubation of bone marrow cells with CH-4 plus complement almost completely inhibits their capacity to form CFU-E without affecting their ability to form GM-CFU. In addition, CH-4 plus complement does not inhibit the capacity of B^2/B^2 lymphocytes to induce a graft-versus-host reaction under conditions where anti-B^2 lymphocyte alloantisera are completely inhibitory. Our results strongly suggest that CH-4 monoclonal antibodies detect a private specificity on a gene product of the B-G locus whose expression is restricted to erythroid stem cells and erythrocytes.

Key words: MHC, erythropoiesis, differentiation marker, B-G locus, monoclonal antibody

The chicken *B* complex [for review see 1] is homologous to the mammalian MHCs [2], such as the H-2 and HLA complexes of mice and humans. H-2 and HLA are known to include several closely linked genes controlling the synthesis of polymorphic cell-surface antigens. In the mouse, the gene products of the H-2D and H-2K regions are found on the surfaces of several different cell types [3], while the products of other loci, especially those found in the H-2I region, have a more limited tissue distribution, being absent from RBCs and most T-cells [4, 5]. The H-2G gene product however, is found on RBCs, but is almost totally absent from WBCs [6, 7]. Unlike most other H-2 regions, which are highly polymorphic, only two alleles are known for H-2G, and it has recently been shown [8, 9] that H-2G gene products are probably not synthesized by erythroid cells but are passively absorbed to the RBC surface and likely represent a component of the complement system.

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The chicken B system controls the synthesis of two classes of antigen, the B-F and B-L antigens, which are homologues of the mouse H-2D (or K) and Ia antigens [10, 11]. The B-F, but not the B-L antigens, are present on RBCs. A third gene of the B-complex, B-G, is found on RBCs but not on lymphocytes [12]. B-G is probably not the homologue of H-2G, since B-G gene products are highly polymorphic [1] and are not obtained by passive absorption from the serum [12].

We have recently succeeded in producing monoclonal antibodies that detect private and public MHC specificities present on erythrocytes of several different chicken MHC types [13; and our unpublished data]. In the present report we describe the spectrum of reactivity of one of these monoclonal antibodies (CH-4) with various cell types of hemopoietic tissue. CH-4 was found to detect a private antigen of the B^2 haplotype found only on cells of the erythroid series, including CFU-E, and presumably a product of the B-G locus.

METHODS

Agglutination

Agglutination assays using monoclonal antibodies were performed as previously described [13].

Rosette-Forming Cell Assay

Direct rosettes were formed by incubating cell suspensions with B^{21}/B^{21} chicken RBCs that had CH-4 monoclonal antibodies coupled to their surfaces using 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide (EDCI) as a coupling reagent. A ratio of 10:1 RBCs to target cells was used, and rosettes were counted following incubation for 180 min at 4°C.

Coupling of CH-4 Monoclonal Antibodies to Chicken RBCs

Chicken RBCs were washed three times with PBS and once with 0.01 M NaCl, 0.35 M mannitol and resuspended as a 10% (V/V) suspension in 0.01 M NaCl, 0.35 M mannitol. A volume containing 25 μg/ml of CH-4 was then added to 100 μl mannitol-salt solution, and 20 μl freshly prepared 10 mg/ml EDCI solution (in mannitol-salt solution) was added. After 2 min at room temperature 1 ml 10% CRBCs as prepared above was added, incubated at 4°C for 1 h, diluted with 9 ml PBS, pelleted, washed once again with PBS, and stored with PBS + 5% FCS. CRBCs with chemically coupled antibody are stable for a maximum of 1 week at 4°C.

Graft-Versus-Host Splenomegaly Reaction

GVHRs were done as described previously [14]. For inhibition of GVHRs [15] WBCs were preincubated with a 1:50 dilution of alloantibody or monoclonal antibody, with or without complement, and the mixture was injected intravenously into day 12 chick embryos, which were killed 7 days later and spleens were weighed.

CFU-E Assay

Erythroid colonies were formed from adult bone marrow cells as described by Samarut and Nigon [16]. For antibody-induced inhibition of erythroid colonies, bone marrow cells were incubated with various concentrations of antibody, with or without complement, washed three times, and plated.

GM-CFU Assay

Granulocyte-macrophage precursors were assayed using a modification of the assay described by Metcalf [17] for growing mammalian cell colonies. Briefly, bone marrow cells were suspended in medium consisting of 0.3% agar (Difco) in MEM supplemented with 10% each of FCS and chicken serum. Serum from a chicken injected intravenously 6 h previously with endotoxin (50 mg LPS per bird) provided an excellent source of GM-CSF [18]. The serum containing GM-CSF was inoculated onto each culture (0.1 ml per dish) after the agar had solidified. Cultures were incubated at 37°C in a 10% CO_2 atmosphere for 6 days. During the scoring, no distinction was made between clusters and colonies.

RESULTS

B^2 Private (MHC) Bearing Cells in Various Hemopoietic Tissues

CH-4 monoclonal antibody, which detects the private antigen of B^2, was chemically coupled to B^{21}/B^{21} chicken RBCs. When these cells were mixed in equal proportions with B^2/B^2 RBCs, strong agglutination was seen within 30 min of mixing. No agglutination was seen following mixing of these cells with five other genotypes of chicken RBCs (Table I). This shows that anti-B^2 monoclonal antibody maintains its specificity for B^2 bearing RBCs following chemical coupling to B^{21}/B^{21} RBCs. The new rosette-forming cell (RFC) assay was then used to determine the percentage of non-erythrocyte RFC bearing the private B^2 antigen in various hemopoietic tissues. Cell suspensions, which were made from the various tissues listed in Table II, were centrifuged twice on Ficoll gradients in order to remove RBCs, mixed with chicken RBCs bearing chemically coupled anti-B^2, and the number of RFC was counted. Adult bone marrow and embryonic spleen were found to contain the highest number of B^2 private antigen-bearing cells; approximately 3–6% of cells from these organs formed rosettes (Table II). Of the remaining tissues tested, only adult spleen and WBCs contained a significant number of RFC, and no RFC were detected in the adult thymus or bursa.

Inhibition of CFU-E Following Pretreatment of Adult Bone Marrow Cells With Monoclonal Anti-B^2 Antibody

CH-4 is an IgM monoclonal antibody which specifically agglutinates B^2-bearing RBCs (Table I) and, in the presence of complement, specifically lyses them. Since the highest percentage of cells bearing the antigen recognized by CH-4 was found in active hemopoietic organs (bone marrow and embryonic spleen), we tested the capacity of CH-4 antibody plus complement to inhibit the formation of erythropoietic and granulocyte-macrophage colonies.

Adult bone marrow cells were incubated in the presence of various concentrations of CH-4 antibody plus complement and tested for their ability to form CFU-E and GM-CFU. CFU-E formation was almost completely inhibited at a 1:1,000 dilution of CH-4 plus complement (Fig. 1). No inhibition of CFU-E formation was seen in the absence of complement (solid square, Fig. 1) or when the bone marrow was pretreated with anti-B^{19} specific monoclonal antibody plus complement (open square, Fig. 1). In contrast to the inhibition of CFU-E with anti-B^2 monoclonal antibody plus complement, no inhibition was seen when the same bone marrow cell suspension was used for GM-CFU formation (Fig. 1). In addition, pretreatment of WBCs with CH-4 plus complement does not inhibit their capacity to induce a graft-versus-host reaction (Table III).

TABLE I. Direct and Mixed Agglutination of Various MHC Genotypes of Chicken RBCs with CH-4 Monoclonal Antibody*

MHC genotype	Direct agglutination titre[a]	Mixed agglutination[b]
B^2/B^2	204,800	++++
B^{13}/B^{13}	< 200	- - - -
B^{14}/B^{14}	< 200	- - - -
B^{15}/B^{15}	< 200	- - - -
B^{19}/B^{19}	< 200	- - - -
B^{21}/B^{21}	< 200	- - - -

*CH-4 was produced following the immunization of CBA mice with B^2/B^2 chicken RBCs [13].
[a]at 37°C, pH 7.5.
[b]CH-4 monoclonal antibody was chemically coupled to B^{21}/B^{21} RBCs and mixed in equal proportions with various genotypes of RBCs.

TABLE II. Percentage of B-G^2 Private (MHC) Antigen-Bearing Cells (Excluding RBCs) in Various Hemopoietic Tissues

	Tissue[a]					
	Bone marrow	Embryonic spleen	Adult spleen	Adult WBCs	Adult thymus	Adult bursa
Expt 1	3.4[b]	5.6	0.6	0.2	< 0.1	< 0.1
Expt 2a	2.6	3.8	0.2	0.1	< 0.1	< 0.1
2b	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

[a]Tissues were from B^2/B^2 chickens for experiments 1 and 2a and from a B^{19}/B^{19} chicken for experiment 2b. All RBCs were removed from the cell suspension by centrifugation on Ficoll prior to rosette formation.
[b]Percent rosette-forming cells; a minimum of 1,000 cells were examined.

TABLE III. Lack of Inhibition of a Graft-Versus-Host Reaction Following Pretreatment of WBCs From B^2/B^2 Chickens With CH-4 Monoclonal Antibody Plus Complement

	Donor WBCs preincubated with:				
	Medium	Complement	CH-4	CH-4 + complement	Anti-B^2 Allo-antibody + complement
Expt 1	66 ± 13[a]	57 ± 10	67 ± 14	59 ± 15	11 ± 2[b]
Expt 2	48 ± 5	51 ± 7	46 ± 10	45 ± 12	9 ± 3[b]

[a]Mg embryo spleen weight ± SD. Embryos were B^{15}/B^{15}.
[b]Positive control, represents complete inhibition of GVHR; ie, these values do not differ from uninjected embryo spleen weights (= 10 ± 1).

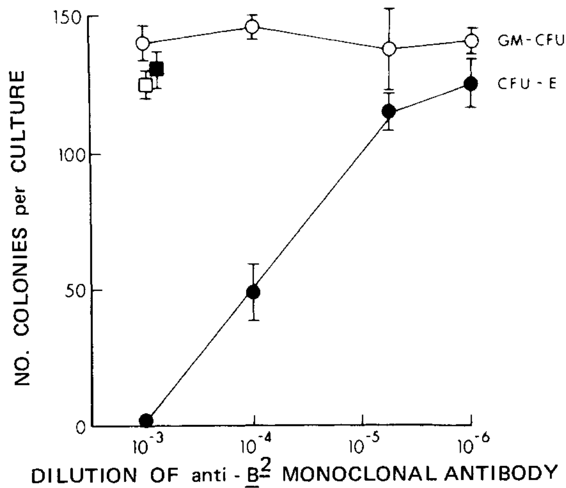

Fig. 1.

DISCUSSION

Our data strongly suggest that CH-4 monoclonal antibody detects a private MHC antigen (B^2), which is restricted to the erythroid line of differentiation. The presence of this B^2 private antigen on erythrocytes was demonstrated using four separate serological tests: direct agglutination [13] (Table I), hemolysis [our unpublished observations], mixed agglutination (Table I), and rosette formation [our unpublished data]. This B^2 private antigenic determinant is also expressed on erythrocytic precursor cells (CFU-E), as shown by the observation that pretreatment of bone marrow cells with CH-4 plus complement completely inhibits their capacity to form erythroid colonies. Furthermore, using the new RFC assay, we found that tissues that are undergoing active erythropoiesis (day 12 embryonic spleen and adult bone marrow) have the most cells that bear the B^2 private antigen detected by CH-4 monoclonal antibody. Other organs not active in erythropoiesis such as bursa and thymus have no cells bearing this antigen. The few RFC found in the adult spleen may be explained by the fact that this organ is capable of undergoing a low level of erythropoiesis, especially in times of stress, and the few RFC found in the blood could be circulating erythroid precursors. The restricted expression of a highly polymorphic MHC antigen in a particular line of cell differentiation suggests that the molecule bearing the antigen may have an important function for that line of differentiation. The best known example of this principle is the expression of Ia antigens in B lymphocytes and certain subpopulations of macrophages and lack of expression in T-cells, erythrocytes, and other hemopoietic cells. These molecules undoubtedly play a role in the immune response. Our finding of the expression of B^2 private antigen, a gene product of the B-G locus of chickens, in erythroid progenitors is consistent with the view that this molecule might play some role in cell interactions involved in erythropoiesis. Thus, it would be of interest to determine how early during the erythroid differentiation sequence (stem cells→BFU-E→CFU-E) that the B-G^2 molecule is first expressed. This information will have to await the development of an assay for BFU-E in the chicken.

To our knowledge, CH-4 monoclonal antibodies identify the first antigenic marker described that is specific to the erythroid line of differentiation. In addition, the development of the RFC assay may allow for the identification and purification of the erythroid precursors that bear this specific MHC antigen marker.

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