# ALLOANTIGENS OF CANINE BLOOD CELLS

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Mammalian blood cells (erythrocytes, platelets, leukocytes) have both common alloantigens and specific allogenic factors [1, 6, 8]. Interconnection between these factors is of great importance to the understanding of the organization of antigenic specificities and the regulation of the immune response. For the latter problem recent data on the presence of antigens specific for B lymphocytes, and evidently connected with genes of the immune response [3, 4, 7], are particularly important. With regard to these problems, information is most complete on man and the mouse, whereas dogs, which are widely used for preclinical modeling of various processes [5], have received comparatively little study.

The object of this investigation was to study the distribution of the various alloantigens on canine blood cells and the possibility that specific antigens of B lymphocytes may exist in dogs.

## EXPERIMENTAL METHOD

Heparinized venous blood from 14 unrelated mongrel dogs was used and erythrocytes, platelets, and lymphocytes were separated from it. In reactions with these cells, 46 hemagglutinating, 42 thromboagglutinating, and 81 lymphocytotoxic sera, obtained by the writers in the course of an exchange connected with joint research during the Second International Working Conference on Immunogenetics of Dogs, were used [5]. The methods of performance of the reactions, of isolating the cells, and of adsorption of the sera were described previously [2].

To separate T and B lymphocytes from a whole suspension of lymphocytes the rosette-formation test was carried out with sheep's erythrocytes, IgG sensitized against them, and mouse complement. After the formation of B-rosettes the lymphocyte suspension was again layered on a density gradient and centrifuged at 400g for 40 min. T lymphocytes were distributed in a ring, and the residue contained B lymphocytes, which formed rosettes. They were freed from erythrocytes by hypotonic shock.

Hemagglutination, platelet-agglutination, and lymphocytotoxic tests were carried out initially with the serum. If a positive reaction was obtained with cells of only one type from a given dog, the serum was exhausted with the cells with which the test was negative, and the original test was repeated. If the repeated test was negative, this meant that the cells used for adsorption contained common antigens with the test cells.

To determine antigens of B lymphocytes the lymphocytotoxic test was carried out initially with a whole suspension of lymphocytes and sera not giving strong reactions (not more than 50% of dying cells, i.e., ++) were selected. The test was then repeated, using separate populations of T and B lymphocytes. Absence of a reaction with T lymphocytes and its presence with B lymphocytes was taken to imply that the serum contained antibodies against antigens of B lymphocytes.

## EXPERIMENTAL RESULTS

Adsorption experiments (Table 1) showed that canine erythrocytes may contain common antigens with platelets (sera Nos. 10 and 113), platelets may contain common antigens with leukocytes (sera Nos. 214, 6M,

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TABLE 1. Alloantigens of Dogs to Various Blood Cells

| No. of serum | Antibodies before adsorption | Specificity of serum | Material for adsorption | Presence of antibodies after adsorption |
|---|---|---|---|---|
| 214 | Lymphocytotoxins | Unknown | Platelets | — |
| 6M | " | DLA-C11 | " | — |
| 33 | " | Unknown | Erythrocytes | + |
| | | | Platelets | — |
| | | | Erythrocytes | + |
| 113 | Thromboagglutinins | " | " | — |
| | | | Leukocytes | — |
| 10 | " | " | Erythrocytes | — |
| | | | Leukocytes | + |
| 602 | Hemagglutinins | DEA 1.1 and 1.2 | Platelets | — |
| | | | Leukocytes | — |
| 06 | " | The same | Platelets | — |
| | | | Leukocytes | — |
| 1340 | " | | Platelets | — |
| | | | Leukocytes | — |
| 1350 | " | N. 3 | Platelets | — |
| | | | Leukocytes | + |
| 1371 | " | Unknown | Platelets | — |
| | | | Leukocytes | + |

TABLE 2. Determination of Alloantigens of Canine B Lymphocytes

| No. of serum | Specificity of serum | Lymphocytotoxic test with various lymphocyte populations | | |
|---|---|---|---|---|
| | | T+B | T | B |
| 23 | Unknown | + | — | ++ |
| 21 | " | +++ | — | +++ |
| 12 | " | ++ | — | +++ |
| KuKa | " | ± | — | ++ |
| 41 | DLA-A8 | ++ | — | ++ |
| 238 | DLA-B5 + B13 | ++ | — | ++ |

and 33) and erythrocytes (sera Nos. 602, 06, 1340, 1350, and 1371), and leukocytes may contain antigens of erythrocytes (sera Nos. 602 and 06) and platelets (serum No. 113). The writers showed previously [2] that leukocytes do not contain the principal antigens of platelets.

It is interesting to note that the strongest antigens of canine erythrocytes (DEA 1.1 and 1.2) are also present on leukocytes and platelets (sera Nos. 602 and 06), whereas weak antigens (2 and 3) are present only on platelets (sera Nos. 1340 and 1350).

Consequently, canine erythrocytes have certain antigens of platelets, but they rarely have antigens of leukocytes. Platelets may carry many antigens of both erythrocytes and leukocytes, whereas leukocytes may have strong antigens of erythrocytes and also, probably, weak antigens of platelets.

The second series of experiments showed that most lymphocytotoxic sera contain antibodies against both T and B lymphocytes of dogs. However, individual samples of sera of unknown specificity (sera Nos. 23, 21, 12, and KuKa) or determining antigens of the 1st or 2nd subloci of the DLA system (sera Nos. 41 and 238) are directed toward antigens of B lymphocytes (Table 2). The presence of such sera makes it possible to type alloantigens of B lymphocytes and to determine the character of their inheritance, and this will be the aim of future experiments.

The results show that dogs can be compared with man with respect to representation of alloantigens on blood cells and the presence of antigens specific for B lymphocytes. If the same similarity of genetic control of different human and canine alloantigens is taken into account [1, 5, 9], the good prospects for the use of dogs in experimental medicine will become more evident still.

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# DIFFERENCES BETWEEN PROPERTIES OF SPECIFIC T SUPPRESSORS AND CYTOTOXIC T LYMPHOCYTES IMMUNE TO ANTIGENS OF THE H-2 COMPLEX


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During immunization of mice with allogeneic spleen or tumor cells a spectrum of subpopulations of T lymphocytes differing in their properties is formed [6]. The possibility of induction of T cells blocking activation of DNA synthesis and generation of cytotoxic T lymphocytes (CTL) in a unidirectional mixed culture of normal lymphocytes (mixed lymphocyte culture – MLC), and their concentration by the adsorption-elution method on a monolayer of allogeneic target cells (TC), was demonstrated by the writers previously [2]. Inhibition of the immune response in MLC may be due to the activity either of CTL eliminating stimulators from MLC [10], or of a special category of T suppressor cells, inactivating reacting lymphocytes [8, 9].

The object of this investigation was to make a comparative study of the properties of T suppressors and CTL formed after immunization of mice with allogeneic spleen or tumor cells. For this purpose specific T lymphocytes were enriched by the adsorption-elution method, by means of which T suppressors can be concentrated 30 times [2] and CTL 6 to 8 times [1].

## EXPERIMENTAL METHOD

Mice of congeneic B10.D2, abbreviated to D2(H-$2^d$), and C57BL/10, abbreviated to B10(H-$2^b$), lines were obtained from the Department of Genetics, Research Laboratory of Experimental Biology and Medicine, "Svetlye Gory." D2 mice were immunized by a single intravenous injection of $9\times10^7$ spleen cells, irradiated with $\gamma$-rays in a dose of 1500 rads ($^{137}$Cs, 740 rads/min), or intraperitoneally with $25\times10^6$ cells of E14 ascites leukemia induced in C57BL/6 (H-$2^b$) mice. In some experiments, 2 days before immunization, D2 mice were given an intraperitoneal injection of cyclophosphamide (CP) in doses of 25-200 mg/kg body weight, dissolved immediately before injection, and hydrocortisone (HC) in a dose of 2.5 mg per mouse. Four days after intravenous immunization, $1\times10^7$ D2 spleen cells were treated with mitomycin C (Calbiochem), in a dose of 50 $\mu$g/ml, in 1 ml Eagle's medium for 30 min at 37°C, washed 3 times, and added to a unidirectional MLC inorder to block activation of DNA synthesis [11] and CTL generation [13]. To suppress DNA synthesis, immune lymphocytes treated with mitomycin C in the ratio of 1.5:1 were added to the reacting lymphocytes, and normal lymphocytes, similarly treated, were added to the control lymphocytes. Suppressor activity was determined by calculating the index of inhibition (II) of DNA synthesis and CTL generation by the formula $[(a-b)/a]\times100$, where $a$ and b stand for incorporation of $^3$H-thymidine or specific liberation of $^{51}$Cr respectively in the control and


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