The Transfusion Behavior of Avian Erythrocytes:
The Lack of Functional Transplantation Antigens in a Nucleated Cell

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THE SURVIVAL of transfused mammalian erythrocytes in a homologous recipient of compatible blood group is an exception to the general phenomenon of host reactivity against genetically divergent foreign cells. A possible explanation for this survival is that, unlike solid grafts, red cells remain in the circulation and therefore may not be exposed to a sufficiently high concentration of the cellular factors which bring about homograft rejection. Another reason for the red cell's failure to incite transplantation rejection immunity could be the absence of histocompatibility antigens. Evidence for this hypothesis is provided, for example, by the failure of injected erythrocytes to heighten resistance to skin grafts in the rabbit or mouse and by their inability to confer tissue tolerance to newborn animals. On the other hand, Barrett's finding that prior inoculation with donor erythrocytes will decrease the growth of transplantable fibrosarcoma in the mouse is in conflict with this concept. The ability of erythrocytes to bestow some immunity to skin homografts in the rat also suggests the presence of transplantation antigens in red cells.

Transplantation antigens have been demonstrated in fractions of nuclear material. A unique characteristic of the mammalian red cell—the loss of its nucleus during maturation—might be responsible for the cell's possible lack of transplantation antigens, and account for its transfusion survival. The persistence of a nucleus in avian erythrocytes offered an opportunity to test this hypothesis. If all nuclear material contained transplantation antigens, and if the mere presence of these antigens inevitably led to homograft rejection, the transfusion behavior of the homologous avian nucleated red cell might show a shortened survival time. It will be shown that no such difference was found when homologous blood group compatible transfusions were carried out.

MATERIALS AND METHODS

Because of the great complexity of blood groups in chickens, the turkey was chosen as the experimental animal. Two strains of turkeys were used: male Beltsville white turkeys and male Bronze turkeys from Colorado State University inbred strain 1-41. The latter group had an 80 per cent coefficient of inbreeding, while the former were offsprings of random matings.

A total of 46 transfusions were given and cell survival determined in 36 instances. Crossmatches between donor and recipient were performed by the saline, albumen and indirect anti-globulin technic. The anti-turkey globulin serum was prepared in rabbits.

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207
with alum-precipitated proteins from pooled turkey sera.\textsuperscript{8} The appearance of antibodies was determined by testing the serum of recipient turkeys at 2-week intervals following transfusion by the above methods against a panel of turkey erythrocytes which included cells from the donor turkey.

For survival studies, 20 to 30 ml. of donor blood was collected in ACD and incubated for 30 minutes with about 3\mu c. ml. of radioactive sodium chromate (Na$_2$Cr$_5$O$_7$).\textsuperscript{9} After the addition of ascorbic acid, 10 ml. aliquots were injected into the donor and each compatible recipient. Since no attempt to separate the various cellular elements was made, approximately 10$^5$ leukocytes were given in each transfusion. Blood specimens were obtained daily or on alternate days for 2 weeks and at 3 to 5 day intervals thereafter, and radioactivity in the whole blood samples was counted in a Nuclear Chicago DS-3 well-type scintillation counter with a model 183 scaling unit; sufficient counts were obtained to limit the counting error to 2 per cent. The hematocrit was determined using an International Capillary Centrifuge and an International Microhematocrit Reader.

Split thickness skin grafts measuring 20 x 30 mm. were performed on the backs of animals using Cannon and Longmire's method,\textsuperscript{10} modified slightly in order to assure total removal of host epithelium from the recipient site. Skin grafts were observed daily; some grafts were biopsied on day 10 and others on day 21 and day 35 after application. Hematoxylin and eosin, reticulum and eosin-methylene blue stains were routinely done on all grafts.

\section*{RESULTS}

The normal autotransfusion red cell survival was determined in 15 experiments in 8 turkeys. The radioactivity in the 24-hour post-transfusion sample was selected as 100 per cent survival, and subsequent samples were calculated as a percentage of this sample. The combined results of these experiments are seen in figure 1.\textsuperscript{6} Under these conditions the Cr$^{51}$ half-life (T$_{1/2}$) for autotransfused blood ranged from 9 to 16 days, with a mean survival of 12.5 days.

Cross-transfusions were carried out in 12 birds, with survival curves falling into two patterns. In the first ("compatible") group, the mean Cr$^{51}$ T$_{1/2}$ survival was 13.1 days (fig. 2), which is not significantly different from the autotransfusion survival pattern. Second transfusions between the same donor-recipient pair were carried out from 3 to 4 weeks after disappearance of the first donor cells. No shortening of the survival time was observed during these second transfusions, nor were antibodies to the donor cells found in the recipient’s serum either after the first or second transfusion. Based on the normal survival of donor cells and the absence of detectable antibody response following two transfusions, these recipients were classified as compatible for the donor cells.

The response to cross-transfusion in the second group was quite different, indicating incompatibility between donor and recipient. After the first transfusion normal cell survival was observed for the first 2 to 4 days, with the slope of the Cr$^{51}$ tagged cell survival curve resembling that of the autotransfusions (fig. 3). This was followed by an accelerated rate of elimination of the transfused cells as manifested by a steeper slope of the Cr$^{51}$ disappearance curve. The mean Cr$^{51}$ T$_{1/2}$ survival time was 7.8 days (range 6-9 days) which is significantly shorter (P < 0.001) than that observed in autotransfusions or in

\*Illustrations in this article courtesy of Medical Audio Visual Dept., Walter Reed Army Inst. of Research, Washington, D. C., U. S. Army Photograph (ANWSO).
COMPATIBLE CROSS-TRANSFUSIONS. Two weeks after these incompatible donor cells had disappeared, a weak incomplete antibody against the donor cells was demonstrable by the indirect anti-globulin technic in 5 out of 6 recipients.

When a second transfusion was given from the same donor to the same recipient, an accelerated rate of destruction was noted in the immediate post-transfusion period (fig. 4), while the normal elimination rate found in the early post-transfusion period of the first incompatible transfusion was not
found. The mean Cr⁵¹ T ½ survival time of 4 days (range 3–6 days) for the second incompatible transfusions was significantly shorter (P < 0.001) than that of the first transfusions. The incomplete antibody became more easily detectable after a second transfusion. Usually, the indirect antiglobulin titer rose from about 1:2 or 1:4 after the first incompatible transfusion, to 1:8 or 1:16 after the second one. When tested by the indirect anti-globulin technic the recipient turkeys' serum now gave positive reactions not only with the donor...
turkeys' cells, but also with some, although not all cells, from a group of turkey cells chosen at random.

Once nucleated red cell compatibility or incompatibility had been demonstrated between a given donor-recipient pair, the response to homografts exchanged between these birds was investigated. About 4 weeks after the last
transfusion, each turkey received a homograft from a blood group compatible
donor, 2 homografts from blood group incompatible donors and an autograft
from its contralateral side. Gross inspection of the homografts and autografts
revealed a pinkish color suggestive of incipient vascularization from the 3rd
to the 5th day postoperatively. Between the 9th and the 12th day, swelling and
dusky discoloration of the homografts had appeared. Induration, hemorrhage
and scaling of homografts became marked after the 13th day; by the 18th day
the rejection was generally complete, while the autografts became indistinguishable from normal skin. The course of homograft rejection was similar in blood group compatible and incompatible donors.

There was considerable variation in the microscopic picture of homografts, depending not only on the time, but also on the site where biopsies were taken. In general, the pattern was characteristic for homograft rejection. The biopsies taken on the 10th and 21st postoperative day showed thinning of the epithelium with disorganization and ballooning degeneration of the cellular elements. The upper layers of the epidermis showed marked cornification, cell boundaries became indistinct and only ghost-like nuclear outlines were visible. An intense cellular proliferation of mononuclear cells which included lymphocytes and plasma cells invaded the entire subepidermis and upper dermis. (fig. 5–9). Varying amounts of edema of the subepidermis was present with marked fragmentation of collagen fibers as well as disruption and separation of elastic fibers. Biopsies taken on the 35th day showed intact epidermis to be again present, perhaps having grown in from the normal skin edge.

Two months after the skin grafts had been rejected in the compatible recipients, a 3rd Cr$^{51}$ labeled blood transfusion was exchanged between the compatible donor-recipient pairs in order to determine the effect of graft rejection upon subsequent blood transfusion. In all of these recipients the Cr$^{51}$
Fig. 6.—Homografts showing ballooning and keratinization of epithelial cells. Twenty-first postoperative day. X 660.

T ½ survival time was still normal, despite rejection of the skin graft prior to transfusion.

While blood groups were compatible in 3 out of 4 birds, homografts were rejected by all 4 Bronze turkeys, despite their 80 per cent coefficient of in-breeding.

DISCUSSION

It is evident from the results obtained in the autotransfusion and compatible cross-transfusion experiments that the turkey erythrocyte has a normal survival in the circulation of a homologous recipient of compatible blood group. Thus, the transfusion behavior of the turkey red cell parallels that of the mammal, even though the avian erythrocyte has a nucleus.

Since transfusion survival is possible despite the presence of nuclear material, it would appear that the lack of a nucleus in mammalian red cells does not necessarily account for their violation of homotransplantation immunity. It has also been demonstrated that the turkeys that had 2 transfusions of homologous red cells with normal Cr⁵¹ T ½ survival time rejected the skin grafts from the same donor. After homograft rejection had occurred, a third transfusion from the same donor still showed persistent blood compatibility with the recipient
Fig. 7.—Cellular infiltration of homograft. Twenty-first postoperative day. X 820.

as expressed by normal Gr21 survival times and negative direct and indirect anti-globulin tests. This host reaction against donor skin in the face of blood group compatibility is in keeping with the individual identity of transplantation antigens as opposed to blood group antigens.9 No conclusions can be drawn concerning homograft rejection in the turkey from these studies. In view of the prior transfusion of leukocytes, it is impossible to state whether we are dealing with a first or second set response, nor was it the purpose of this study to investigate these aspects beyond establishing homograft incompatibility despite nucleated red cell compatibility.

The survival of transfused nucleated compatible erythrocytes is in contrast to the host response to nucleated cell precursors present in bone marrow, which characteristically evoke transplantation immunity in the homologous host. What then accounts for these differences in reaction? Blood group incompatible erythrocytes will be coated by serum antibody, but blood group compatible erythrocytes escape transplantation immunity mechanisms. Several explanations may be suggested. Transfused erythrocytes exist as circulating cells and thus may not concentrate their antigenic action upon a localized group of lymph nodes. Bone marrow transplants on the other hand, even when injected intravenously, find regional localization through the cells’ “homing instinct”.

12
The rejection of non-circulating cells may thus be the result of more marked stimulation of regional lymph nodes (afferent stimulus); however, an alternative explanation, i.e., the possibility that fixed foreign cells are more accessible to the immune response of the stimulated lymphoid rejecting mechanism (efferent response) must also be considered. The special circumstance of the transplanted red cell may be related to the characteristically cellular, rather than humoral nature of transplantation immunity, and account for the fact that circulating red cells are susceptible to only one type of immune response.

As mentioned above, the absence of transplantation antigens would also explain the survival of the transfused cells. Most of the evidence for this hypothesis stems from studies with mammalian red cells. For avian erythrocytes some disagreement exists between the experimental findings of Billingham et al., who indicated that chicken red blood cells are not effective in inducing tolerance, and that of Cannon et al., who reported that adult chicken RBC suspensions were the most effective blood elements in inducing tolerance. If transfusion survival truly reflects the absence of histocompatibility antigens, rather than the special circumstances of a circulating cell, our findings would support Billingham’s suggestion that erythrocytes are devoid of these antigens. If this be the case, it remains a puzzle why these antigens, which are associated

Fig. 8.—Wilder’s reticulum stain of autograft on 10th postoperative day. X 216.
Fig. 9.—Wilder’s reticulum stain of homograft, showing fragmentation and disorganization of fibers on 10th postoperative day. X 102.

with nuclear material and are found in the nuclei of bone marrow erythropoietic elements, would be lacking from the mature circulating nucleated avian red cell.

Several well recognized immunologic phenomena are illustrated in these experiments. The removal pattern of incompatible red cells showed the characteristic primary response, consisting of a post-stimulation (i.e., transfusion) latent period where antigen remains in the circulation, followed by rapid elimination of antigen (i.e., red cells), and a secondary response with an earlier or even immediate onset of the accelerated rate of removal of red cells. A similar pattern has been observed by Mitchinson who employed the Cr⁵¹ technic to illustrate the effect of persistent antigen on acquired tolerance.

The development of an incomplete agglutinin is undoubtedly a manifestation of the presence of blood groups in the turkey. The usefulness of the rabbit anti-globulin serum becomes apparent in that it was the only technic, aside from the Cr⁵¹ survival studies, which detected blood group incompatibility. The Cr⁵¹ technic is considerably more sensitive in detecting blood group incompatibility than the demonstration of antibodies. It is known that in the absence of preformed antibody the first antibody to appear in the circulation will combine in antigen-antibody complexes which will be removed from the
blood stream by the reticulo-endothelial system. The bend in the Cr⁵¹ survival curve thus, in all likelihood, corresponds to the first appearance of antibody and antedates the positive serologic tests of incompatibility.

**Summary**

1. Autotransfusion and cross-transfusion experiments in the turkey reveal that the avian erythrocyte, despite the presence of a nucleus, survives normally in homologous recipients of compatible blood groups.
2. Skin homografts were rejected without apparent relationship to blood group compatibility.
3. Evidence for blood groups in the turkey is presented. While no naturally occurring iso-agglutinins were found, stimulation led to the appearance of incomplete antibodies.

**Summario in Interlingua**

1. Experimentos de autotransfusion e de transfusion cruciate in gallos de India indica que le erythrocyto avian, in despecto del presentia de un nucleo, supervive normalmente in recipientes homologe de compatibile gruppos de sanguine.
2. Homograffos de pelle esseva rejicite sin apparente relation con le gruppos de sanguine representate.
3. Es presentate evidentia pro le gruppos de sanguine in le gallo de India. Durante que nulle iso-agglutininas de occurrentia natural esseva incontrate, stimulation resultavas in le apparition de anticorpore incomplete.

**References**


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