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The Origin of the Natural Antibodies for Human Blood Group Antigens.

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It is a generally accepted fact that new antigens arise in animals during evolution through mutations. We shall here tentatively assume that the genes which control antigenic specificity undergo mutations, not only in the germ cells, but also in the somatic cells of each individual, and that they do so perhaps at the very same rate. On the basis of this assumption, we may then ask how such mutations would manifest themselves.

In man there is one blood group which is characterized by three genes: A, B, and O. These are mutually exclusive because they occupy the same locus on the chromosome; i.e. they are allelic genes. Since each individual carries two sets of chromosomes, the genotype of each

individual may be written as AA, AB, BB, AO, BO and OO. The genes A and B elaborate each an antigen which is present in the red blood cells and which differ in their antigenic specificity.

Let us now assume that the allelic genes A, B and O can mutate into each other and that their mutation rate in the germ cell is sufficiently high to lead, in the absence of selection, to a distribution which is not too far from the equilibrium, and let us further assume that these genes mutate in the bone marrow cells of each individual at the same rate as in the germ cells. We then obtain the following:

(1) The mutation rate in the germ cells must be about one in a million or the rate per year must be about one in thirty million.

(2) There are in man about 3×10^{11} cells in the bone marrow which produce red blood cells at the rate of about one per day. Each of these red cells lives for about 100 days. According to our assumption, within one year there will be produced one in thirty million; i.e. 10^4 mutant bone marrow cells of the "wrong" antigenic type which will produce one mutant red cell per day, each of which will live 100 days. Thus an individual of the genotype (OO), for instance, will have

10^6 red cells of the antigenic type (OA) and ~~of the type~~ (OB) present in his blood. Similarly, the genotype (OA) or (AA) will have 10^6 red cells of the antigenic type (OB) and (BA) in his blood.

If we inject 10^6 sheep red cells into a rabbit, the rabbit responds by producing an appreciable amount of antibody against sheep red cells. Similarly we may expect that 10^6 red cells of the antigenic type (OA) or (OB) may produce an appreciable titre of antibody against antigen A and against antigen B in an individual of the genotype (OO). Or to put it more generally, that is, we may expect to find ~~in man natural iso~~ antibodies to occur naturally in the A.B.O group ~~agglutinines~~ for the antigen A ~~and for the antigen~~ or B in every case when the genotype does not contain the allelic genes A or B. Of course, we would not expect any antibody to be produced in response to antigen if the individual was exposed to this antigen in substantial quantity during his embryonic development, and therefore no natural iso-agglutinines can be expected for an antigen which is elaborated by a gene that is contained in the genotype.

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This is, of course, what is actually found and these naturally occurring antibodies are called natural iso-agglutinines. The titre of the naturally occurring anti-A and anti-B iso-agglutinines is not very high and it can be increased by iso-immunization; i.e. by injecting the red cells of the antigen type A or B. In large animals, such as the chimpanzee, the gorilla, the cow, and the horse, we find naturally occurring iso-agglutinines for certain blood antigens, and in addition also iso-immune agglutinines for certain other blood antigens in which there are no naturally occurring iso-agglutinines present. In the latter case, one might postulate that the mutation rate to the wrong antigen is too low to produce an appreciable number of red blood cells of the wrong antigenic type.

The number of mutant red cells will be low not only when the mutation rate is low but also when the size of the animal is small, and indeed we find that none of the small animals has naturally occurring iso-agglutinines against the blood antigens, but that immune iso-agglutinines can be readily produced by injecting blood of the wrong antigenic type in monkeys, chickens, rabbits, rats, and mice.

~~Isoglutinines and~~ Somatic Mutations and natural antibodies for human blood groups antigens.

It is a generally accepted view that new antigens arise in animals through mutations during evolution. We shall tentatively assume that the genes which control antigenic specificity undergo mutations not only in the germ cells but they also and perhaps at the very same rate -- undergo mutations in the somatic cells of each individual animal. Therefore, we must now ask how such mutations would manifest themselves.

In man there is a blood group characterized by three genes, A, B, and O, which are mutually exclusive because they occupy the same locus on the chromosome; i.e. they are allelic genes. Since man carries from each chromosome (except the sex chromosome) two homologues, the genotype of each man can be written as AA, AB, BB, AO, BO, or OO. The genes A, and O elaborate each compound which is antigenic but is different antigen specificity, whereas for the purposes of this discussion we may assume that the gene OO does not give rise to an antigen.

We shall now assume that the genes A, B, and O can mutate into one another, and we shall further assume that the mutation rate is

just high enough to produce, in the absence of any selection, in thirty million years a stationary mutation equilibrium in which all three genes are present in appreciable ~~amounts~~ (fractions of the population). And finally we shall assume that these genes mutate in the bone marrow cells of each individual at the same rate at which they mutate in the germ cells. We then obtain the following picture:

1.) The mutation rate in the germ cells must be about one in a million per generation or one in thirty million per year.

2.) There are in man about 3×10^{11} cells in the bone marrow which produce red cells at the rate of about one per day each. Of these at least one in thirty million will be mutants, ~~this means~~ of a given type. (i.e. $\sim 10^4$ cells) will ~~divide~~ (will each produce) these mutant bone marrow cells one red cell per day since a red cell ~~has~~ bears about 100 days ~~so~~ a man of the genotype (OO) will have 10^6 red cells of the type (OA) ~~out of the~~ and (OB) present in his blood. Similarly a man of the genotype (OA) or (AA) will $\sim 10^6$ red cells of the type (BA) or (OB). And a man of the type (OB) or (BB) will have $\sim 10^6$ cells of the type (AB) or (OA).

1A

~~Insert, Page 2~~

~~Now if we have an~~ In a man indivual ~~of the genotype 00 one in thirty~~ has 30 million cells in ~~of~~ his bone marrow ~~cells~~ of which i.e. 10^4 bone marrow cells, will produce a mutant type red cell AO, and another 10^4 bone marrow cells will produce a mutant red cell of the type BO. Since a red cell lives about 100 days, we ought to have 10^6 red cells of the type AO and 10^6 red cells of the type BO among a total of 3×10^{13} cells of the type OO. This is presumably enough to evoke the production of agglutinines against antigens A and B in the OO genotype. At least one should think this is enough on the basis of the experience that 10^6 sheep red cells injected into a rabbit produces a potent agglutinating antibody.

INSERT on page 7 A B O , M N

Assuming that in these smaller animals the allelic blood group genes mutate into one another with the same frequency which we have assumed for man for the A B O group because the number of bone marrow cells is much smaller than in man, the mutant red cells may be produced in a number which is too low to evoke an antibody response. Something like this might be the explanation of why natural iso-antibodies can be found only in the larger animals such as

and not in the smald animals like

which are nevertheless quite capable of forming immune iso-antibodies against the blood antigens of their own species.

The A B O Group continued

(1) Take old blood

(2) By injecting type of blood into an individual who does not carry the corresponding gene (isoimmunization), one can greatly increase the titre of the circulating anti-antibodies, and the same holds true for the antigen B. The production of such iso-immune antibodies would be expected even if the mutation rate of the genes A, B, and O into each other were as very low as zero, but in that case there would be no natural iso-antibodies. Such a situation seems to hold in a number of smaller animals, such as

in which iso-immune antibodies can be produced with good efficiency but there are no corresponding naturalized antibodies. In another blood group in man, where there are two allelic genes M and N, there are no natural iso-antibodies, and only in rare individuals is it possible to evoke the appearance of immune iso-antibodies against M and perhaps never against N. This must be contrasted with the fact that both the antigens N and M are good antigens for the rabbit which produces antibodies against either. This leads us to assume that alleles M and N mutate into each other with fairly high frequency in the somatic cells, and that no anti-

bodies are formed in the adult because the wrong antigen was present during embryonic development at such a high titre as to make it subsequently difficult or impossible to form immune iso-antibodies against these antibodies. It should be possible to decide by an experiment whether this interpretation is correct. This experiment would run as follows:

We immunize a rabbit with ~~M M~~ NN type of blood and absorb out the wrong undesired antibodies with ~~M N~~ MM type blood. If we add this anti-~~M~~ N antibody to (M N) type of blood in the presence of complement most of the cells should lyse ~~xx~~ but a certain number of mutant cells which are of the type (M M) will remain unlysed. If these are centrifuged down and viewed under the microscope, there ought to be present a reasonable number, ~~and~~ most of them should be ~~lysed~~ seem to lyse if we add anti- M M rabbit serum to the cell suspension.

A third blood group in man is represented by the two allelic genes Rh and rh. Again there are no natural iso-antibodies. Rh is a good antigen in the rabbit while rh seems to be no antigenic. However, only a fraction of the human individuals who are of the type rh rh can be immunized by injecting them with Rh Rh type of blood.

Most Rh negative individuals do not respond to the production of anti-Rh iso-agglutinine when injected with Rh Rh or Rh rh type of blood. It may again be assumed that this situation is brought about by a too high mutation rate of the ~~xxxxx~~ rh gene to Rh ~~so that there is~~ and a ~~partial loss~~ an impairment

Therefore most indiviuals have lost during their embryonic development the ability to produce anti-Rh iso-agglutine after birth.

general type OB or BB carries natural antibodies against the antigen A. Insert Quite similarly among chimpanzees and gorillas an individual carries natural antibodies against the antigen which is not represented by the corresponding allelic gene in the general type of the individual. If this view is adopted, then one is forced to explain the fact that chimpanzees possess only two of the three blodd types, and gorillas possess only two of the three blood types by assuming that fairly late in evolution some mutation occurred in an individual chimpanzee which has a blood type and that this chimpanzee happened to mate with another chimpanzee of the type . (This could be checked through the distribution of the two blood groups among chimpanzees that can be found today.) Or else we may assume that the genes B are originally present in a fairly small isolated chimpanzee population and got lost through genetic drift.

Since we have two blood groups A in man, A_1 and A_2, we may refine our analysis by analyzing the following facts:

1. General types who produce antigen A_2 can be stimulated to produce antibodies against A_1, but the reverse is only rarely possible, indicating that A_1 and A_2 have an antigenic structure

in common but that A_1 possesses an antigenic structure which A_2 does not possess.

2) Individuals of general type BB produce natural antibodies both against A_1 and A_2.

3) While practically all the individuals of the general type BB produce natural antibodies against A_1, only one-quarter of the individuals of the general type A_2B produce natural antibody against A_1, and of the individuals of the general type A_2A_2 only a few per cent produce natural antibody against A_1.

On the basis of the views here presented, one would say that mutations from A_2 to A_1 are rare compared to mutations from B to A_1. On this basis we would then say that the A_2B type of individual (with half as many B alleles as the BB type of individual) produces half as much A_1 antigen than the BB type individual, and that therefore fewer of such individuals have an appreciable quantity of natural antibody against A_1.

A_1 is the more frequent type.