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Review article

Effects of genetic, epigenetic, and environmental factors on alloimmunization to transfused antigens: Current paradigms and future considerations

Influence des facteurs génétiques, épigénétiques et environnementaux sur l'allo-immunisation au cours de la transfusion : progrès réalisés dans leur connaissance et futures directions de recherche

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Abstract

Transfused red blood cells, platelets, or coagulation factors have the capacity to induce alloantibodies, which once formed, can be a clinical barrier to future transfusion therapy and/or transplantation. Large observational studies over the last 50 years have characterized some of the general properties of transfusion induced alloimmunization, which appear to vary to a considerable extent from what is generally observed for human responses to other immunogens, such as microbial pathogens and vaccines. Transfused cells and factor only induce immune responses in the minority of recipients. There are data to suggest that differences in the unit may play a role. However, there are clearly differences in recipient biology, as once a recipient makes one antibody they are much more likely to make additional antibodies; indeed, recipients have been categorized as “responder” and “non-responder” by the field. Recent mechanistic studies have begun to define potential causes for such differences in alloimmunization from patient to patient, but much progress needs to be made to understand how, why, and in whom alloimmunization occurs. This review gives a general background on immunology in the context of transfusion, summarizes recent progress in the field, and discusses future directions for exploration. Particular attention is paid to the general concept that the human immune system is melded by the wide range of antigens encountered in our environment, and that the effects of such on the immune system may have a profound effect upon response to transfused cells. © 2012 Elsevier Masson SAS. All rights reserved.

Keywords: Alloimmunisation; Transfusion; Red blood cells; Environment; Responders

Résumé

Les globules rouges, plaquettes ou facteurs de la coagulation ont la capacité d'induire la production d'allo-anticorps lorsqu'ils sont transfusés, ces anticorps devenant alors une barrière pour d'autres transfusions ou transplantations. Au cours des 50 dernières années, de larges études observationnelles ont montré que l'immunisation post-transfusionnelle était différente de celle observée vis-à-vis d'agents infectieux ou de vaccins. L'allo-immunisation n'est observée au cours de la transfusion que pour une petite minorité de receveurs. Certaines études suggèrent un rôle de la constitution des produits sanguins. Il existe cependant clairement un impact de la physiopathologie du receveur. En effet, les receveurs ont pu être classés en répondeurs et non répondeurs. Des études récentes ont permis de mieux comprendre ces mécanismes receveurs « dépendants », mais le champ d'investigation est encore vaste pour comprendre totalement l'ensemble des facteurs mis en jeu. Cette revue décrit l'état de l'art des données immunologiques en contexte transfusionnel, résume les progrès réalisés dans ce domaine et discute les futures directions de recherche.

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Une attention particulière est apportée au fait que l'organisme est en permanence exposé à une diversité importante de stimuli antigéniques en provenance de l'environnement, et que la réponse immunologique à ces stimuli peut interférer sur la réponse aux globules rouges transfusés.

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Mots clés : Allo-immunisation ; Transfusion ; Globules rouges ; Répondeurs ; Environnement

1. Programming of the immune system in the context of the broader environment

It is a common oversimplified misconception that the human immune system evolved to recognize and react to foreign antigens but not self-antigens. Although straightforward in concept, and consistent with general themes, this view is simply incompatible with observed immunobiology. As an illustrative exercise, consider the plight of an animal with an immune system that recognized and reacted to all foreign antigens. To be sure, such a system would be successful in preventing disease from infecting pathogens. However, an animal with such a system would mount an immune reaction to any xeno-antigens found in food, and rapidly find itself incapable of finding sustenance against which it did not have a potentially dangerous immune response. Perhaps, more problematic would be immune responses that essentially every pregnant female would make against paternal antigens expressed by fetal tissues. On top of this are the more complicated issues of neo-antigens developed in specialized tissues that are not present in the genome of the whole organism (e.g. recombined genes as a result of crossing over during meiosis in gonadal tissues, recombined T cell receptors, and even recombined immunoglobulins themselves). These problems are by no means just theoretical; indeed, examples of the above predicted outcomes can be found in food allergies, hemolytic disease of the fetus and newborn, neonatal alloimmune thrombocytopenia and immune based infertilities (due to either autoimmunity or alloimmunity to paternally derived antigens expressed by the fetus) [1–3]. However, such diseases result from the breakdown of the normal state of immune regulation, which is to prevent response to food (through the well described phenomenon of oral tolerance), to avoid immunity to fetal antigens through the specialized and tolerogenic environment of the uterus and immunomodulation of the mother, and to avoid reproductive infertility through tolerogenic properties of the testes and ovaries [4–7]. Thus, the immune system has had to evolve the capacity to mount a vigorous response against some, but not other, foreign antigens.

The basis by which the immune system has the capacity to not respond to some antigens (i.e. food and fetal antigens) but to rapidly react to microbial pathogens has been a matter of intense study for the past several decades. A seminal breakthrough in this field was the identification of innate immune machinery that had the capacity to specifically detect antigens associated with microbes and to direct adaptive immunity to respond in such cases. Perhaps, best known are the family of Toll-like receptors (TLRs) that have the capacity to recognize a wide variety of chemical moieties found on microbes but absent from animals and plants (e.g. double stranded RNA, lipopolysaccharide, etc.) [8]. However, the explosion of understanding in TLR biology

was just the beginning; more recently, an entire panoply of gene products that detect microbial motifs and instruct immunity have been described (e.g. NOD like receptors [9]). In aggregate, receptors that recognize foreign motifs are called pattern recognition receptors (PRRs) and constitute a vast surveillance system that functions to detect and respond to bacteria, viruses, parasites, and fungi when they exist in what should otherwise be normally sterile anatomical compartments.

The general paradigm that microbial chemical motifs instruct adaptive immunity is elegant both in its mechanistic predictions and in its simplicity. However, anomalous to this model is the observation that healthy gastrointestinal systems contain a vast array of bacteria, viruses, archaea, and eukaryotes [10]. Since the advent of the microscope, it has become abundantly clear that microbial life is ubiquitous in the human environment, with a vast and extensive variety of species. Early analysis of the microbe-human interface focused on identifying specific microbes responsible for human disease; a search that continues into present time. Subsequently, it became appreciated that in addition to being responsible for many diseases, microbes are also required for human health in the form of our natural flora. Complex microbial communities live within our gut, on our mucosal surfaces, and on our keratinized skin. The complexity of our natural flora has been grossly underestimated as a result of our only observing that which can be cultured by existing methods; recent genetic analysis of gut contents suggests that much of our gut flora has never been identified by current culture techniques [10]. In addition to bacteria, there are numerous viruses that exist in humans with no link thus far to any manner of disease. Whether such viruses are beneficial is unclear, but they are clearly intimately involved in human biology, existing not just on our surfaces, but within our cells (and in some cases within the human genome itself). Thus, in the healthy state, human biology contains a vast and complicated interface with a panoply of microbial entities. Although humans are clearly distinct organisms from the microbes that live within and on us, an astounding degree of mutual co-dependence has evolved. In addition to its role in fighting off microbes known to cause disease, the immune system plays a central role in maintaining a healthy relationship with the resident flora. It is well known that severe dysfunction or lack of an immune system, either due to congenital or acquired immunodeficiency, can result in death due to infection by opportunistic pathogens. In some cases, otherwise non-pathogenic microbes cause severe disease in severely immunosuppressed patients. Thus, the immune system must achieve a very delicate balance in its management of environmental microbes. Truly dangerous microbes need to be eliminated while beneficial microbial populations must be encouraged to reside within specific anatomical compartments without being allowed to either overgrow or invade other anatomical compartments. This

constitutes a daunting task indeed for the immune system, where either hyperactivity or hypoactivity can lead to pathology and death. In any case, managing this balance has a profound effect on the ultimate programming of the immune repertoire and general immune responsiveness. Herein, we consider how such adaptation may affect alloimmunization to transfused cells.

2. Unique properties of immunization to red blood cells, platelets and coagulation factors

The entire process of alloimmunization is in itself a curious immune phenomenon, which is largely an iatrogenic product of modern medicine. Indeed, the only obvious source of alloantigenic exposure in the natural state (i.e. in the absence of organ transplantation or transfusion), would be during reproduction; a setting in which a strong selective pressure exists to avoid alloimmunity. Nevertheless, alloimmunization is an extremely robust process in the context of transplantation, and likely represents the triggering of responses that evolved as part of protective immunity to microbes.

Direct presentation by foreign major histocompatibility complex (MHC) on donor tissue(s) causes rapid and robust recipient anti-donor responses. This is decreased in human leukocyte antigen (HLA) matched transplants, although “matched” donors typically still have foreign MHC molecules. In the case of HLA identical transplants (from matched siblings), differences in HLA sequence are eliminated; however, minor histocompatibility antigens persist. Minor histocompatibility antigens are polymorphisms in the peptides that are presented by the MHC, and they occur in great numbers between any two individuals who are not identical twins [11]. For example, while two individuals may encode identical MHC molecules, they will differ in non-synonymous SNPs (single nucleotide polymorphism) throughout their genome. Because the T cell receptor recognizes an MHC presenting a given peptide, any difference in peptide presented by two different people (even on the same MHC) will nevertheless activate a T cell as an antigen.

Red cell blood antigens often vary by single amino acid differences between donor and recipient (i.e. Kell, Kidd, Duffy, SsU, etc.) [12]. This is also the case for non-HLA based platelet antigens (i.e. HPA₁₋₁₅) and for patients with hemophilia A and B (when the mutation results in missense mutations [13]) who receive replacement factor VIII or IX, respectively. Thus, in this context, antigens in transfused cellular and factor products tend to be minor histocompatibility antigens. In many ways, minor alloantigens are presented similar to microbial antigens. Specifically, for both microbial antigens and minor histocompatibility antigens, one’s own T cells recognize foreign microbial peptides in the context of one’s own MHC, which is the same MHC to which the T cells are educated in one’s own thymus. Thus, from a biochemical standpoint, there is no difference between a minor histocompatibility antigen and a microbial derived peptide antigen, except for the extent of difference from self.

Unlike immune responses to minor histocompatibility antigens, which are relatively weak, immune responses to pathogenic microbes approaches 100%. The particular immune response may not confer effective protection to the recipient,

but a response typically occurs. Indeed, monitoring serology has been a standard method of diagnosing infection and monitoring exposure of both the individual and a population to pathogens for over a century. In immunocompetent recipients, the serological response rate to infection with human immunodeficiency virus (HIV), hepatitis C virus (HCV), HBV (hepatitis B virus), etc., approaches 100%. Likewise, the generation of “naturally occurring” antibodies against A and B antigens in the ABO system, which are formed as responses to resident flora, approaches 100%. In this context, it is a meaningful difference that alloimmunization rates to blood group antigens, human platelet antigens, and factors VIII and IX are quite low by comparison. Despite multiple transfusions from random donors, and thus exposure to a large number of polymorphic red blood cell antigens, the rate of alloimmunization to red blood cells is only 3–5% [14]. Likewise, alloimmunization to human platelet antigens is approximately 8% and alloimmunization rates of hemophilia patients with missense mutations in factor VIII or factor IX is 1–4% [15,16].

3. Extent of immune barrier and MHC variability

One important hypothesis is that it is the very small extent of the immune barrier (e.g. a single amino acid) that is of itself the main reason for the relatively low rates of alloimmunization observed for blood antigens in general. Indeed, the only protein red blood cell antigen that is routinely present in donors but absent in recipients is RhD. Notably, alloimmunization to RhD is substantially higher than any of the other blood group antigens, approaching 80% in historical control studies, although more recent clinical studies have estimated somewhat lower rates of alloimmunization of RhD⁻ individuals in response to RhD⁺ red blood cells in different clinical settings [17–19]. The reason for the discrepancy is unclear, but in either case, alloimmunization rates far exceed that seen for other blood group antigens. Similarly, patients with hemophilia who are missing large portions of the factor VIII or factor IX protein make inhibitory antibodies at a substantially higher rate than patients with a single amino acid mutation (~35% vs. 4% and 30% vs. 1%, respectively) [16]. One particular exception to this rule, which upon initial examination appears to reject the hypothesis, is that the large number of patients of African descent who lack Duffy antigens on red blood cells do not have increased rates of alloimmunization to Fy antigens. However, in this case, the lack of Fy antigens on red blood cells is typically due to a mutation in the GATA box of the promoter that drives expression of the Duffy gene (*DARC*), resulting in loss of expression of Fy antigens on red blood cells but not other tissues (i.e. kidney, blood vessel, and brain) [20]. Accordingly, both T cell tolerance (during thymic education) and B cell tolerance (in the periphery) can still occur to Duffy antigens in such individuals. Thus, unlike genetic deletions in which the gene product is absent, Fy antigens remain “immunological-self” in most (Fy^{a-}, Fy^{b-}) patients, preventing anti-Fy antibodies through normal immune tolerance mechanisms. Other less common instances of actual genetic deletion of blood group antigens have been observed (e.g. actual deletion of Duffy, deletion of glycophorin A [Ena⁻], deletion of Kell

[Kell null], etc.) [12]. However, given the very small number of known patients in this category, and the fact that they typically only come to the attention of health care providers as a result of antecedent alloimmunization, no reported data are available on the frequency of alloimmunization in such groups.

The importance upon rates of alloimmunization of the single amino acid difference that constitutes the vast majority of blood group antigens becomes clear in the context of genetic diversity of the MHC in humans. MHC molecules, which present processed peptides to T cells, are amongst the most variant products encoded by the human genome. The variability in the MHC directly alters the sequence of peptides that can fit in the presentation pocket of the MHC molecule. Accordingly, when a group of different individuals is infected by the same pathogen, and is thereby exposed to the same foreign proteins; although each person's immune system will clear the pathogen, the particular viral T cell antigens that are recognized will vary from person to person as a result of which peptides fit into that person's particular MHC molecules.

A virus will encode multiple non-human proteins that vary highly from self-proteins; accordingly, any given person's MHC will find peptides it can present (albeit different peptides for each person). In contrast, because most foreign blood group antigens constitute a single amino acid difference between donor and recipient, the ability of the recipient's immune system to respond depends upon whether or not that individual's MHC can present a peptide containing the variant amino acid. Thus, unlike an antigen with numerous foreign peptides, it is predicted that a subset of recipients will not be capable of responding to a given blood group antigen if no peptide containing the variant amino acid fits into the MHC pocket of the recipients' particular MHC type. The above prediction is made in the context of classical immune response science carried out in inbred mice, which allows the isolation of particular MHC types and repeated analysis of their function. Different strains of mice with distinct MHC types each mount strong immune responses to antigens that are highly foreign. In contrast, when an antigen is given that is highly similar to a normal mouse protein, but which varies only by a small number of amino acids, then some strains of mice mount robust immune responses whereas others do not [21,22]. Subsequent congenic studies (breeding the MHC from one strain onto the genetic background of another) demonstrate that this "responder" vs. "non-responder" status is typically dictated by the MHC type. Indeed, this approach of following genetics of immune response allowed the initial genetic mapping and identification of the MHC itself.

The hypothesis that alloimmunization to red blood cell antigens is controlled by MHC type has been tested by analyzing alloimmunization rates of transfused patients as a function of a given patient's HLA type (HLA type is determined by particular variability in MHC). Indeed, it has been observed for Kidd, and Duffy antigens, that particular HLA types are predisposed to alloimmunization whereas other types appear to have alloimmunization rates that are distinctly lower, or in extreme cases absent [23–26]. These data are consistent with the hypothesis that variability in the MHC (e.g. HLA type) affects the ability to become alloimmunized due to the relative efficiency with which

a peptide containing foreign sequence can fit into the pocket of an MHC molecule and thus represents an epitope that can be recognized by a T cell. In humans, it is not possible to isolate the MHC as the only difference between individuals; thus, such studies always suffer from the caveat that genetic differences in close proximity to HLA – or at least in linkage disequilibrium from it – and not the MHC itself may be responsible for the differences in alloimmunization. However, the identical trends are seen between humans and mice (i.e. as antigens become closer and closer to self-proteins then immune responsiveness transitions from universal to being restricted to expression of certain MHC variants in the recipient). As above, in mice, one can demonstrate that this is due to the MHC itself, and not a correlation to adjacent genes. Given the phenomenological alignment of these two systems, the causal confirmation in mice carries weight in the interpretation of the human data, in which the logistics of testing causality are not ethically feasible.

While one cannot isolate the MHC differences in people (while controlling for all other genetic determinants), one can certainly test an additional prediction of the MHC restriction hypothesis. In particular, any blood group antigen that varies widely between donor and recipient (e.g. much more than just a single amino acid), should induce alloimmunization independent of MHC type. As above, it is important to note that RhD is present on D⁺ donors and absent on D⁻ recipients. RhD is not completely foreign to D⁻ recipients, as there is substantial homology between RhD and RhCE; nevertheless, unlike the other protein blood group antigens, there are a large number of amino acid sequences contained within RhD that are absent from RhD⁻ individuals. No association between alloimmunization to RhD and HLA type have been observed [27,28], although it is worth noting that resolution of HLA typing was less sophisticated at the time of these studies. Severity of alloimmunization and hemolytic transfusion reactions during transfusion of RhD⁺ red blood cells into patients with anti-D has been linked to HLA type [29]; however, this is a distinct biological process from afferent alloimmunization and is likely due to other non-MHC immune genes known to be contained with the HLA. Thus, the apparent lack of HLA restriction for RhD indicates that HLA restriction biology is not a general property of alloimmunization to antigens carried on red blood cells. Rather, these data are further evidence consistent with the hypothesis that reduction of antigenic differences to single amino acids results in restriction of immune responses based upon the ability of variant peptides to be presented by a given MHC.

It is essential to note that the ability of a given MHC sequence to present a peptide containing a variant amino acid from a blood group antigen will be different for every blood group antigen. For example, there is no reason to predict a priori that a particular MHC type, which is incapable of presenting peptides containing the methionine vs. threonine seen in K vs. k antigens, will likewise have difficulty presenting peptides containing the glycine vs. aspartic acid seen in Fy^a vs. Fy^b antigens. Thus, while HLA type and MHC sequence of a particular individual appear to regulate their propensity to become alloimmunized to a given red blood cell antigen, it does not follow that general "responder" vs. "non-responder" status (i.e. likelihood of

becoming alloimmunized in general) will be predicted by HLA type.

Finally, it is important to consider what role the above paradigm plays in understanding relative immunogenicity of blood group antigens. It is an interesting observation that in the context of blood group antigens where donors and recipients differ by only a single amino acid, there is a wide range of different immunogenicities (some antigens induce immune responses much more frequently than do others). This isn't simply due to genetic frequencies where antigen negative individuals are more common in the recipient pool and antigen positive individuals are more common in the donor pool, as differential immunogenicity persists after correcting for frequency of exposure [30]. Moreover, this difference persists (albeit with a slightly different hierarchy) after taking into consideration that antibodies against some blood group antigens are likely not detected due to rapid evanescence [31,32]. Overall, the empirical evidence indicates that despite all consisting of a single amino acid differences, some blood group antigens are simply more immunogenic than are others.

Making sense of differential immunogenicity among blood groups despite having similar molecular variation (i.e. a single amino acid) can be challenging until one considers the issue of HLA restriction raised above. The K antigen from the Kell system is the most immunogenic blood group antigen consisting of a single amino acid alteration. Likewise, of the systems studied, alloimmunization to the K antigen has the lowest HLA restriction and appears capable of inducing an alloimmune response in recipients with a wide variety of different MHC types [23,24]. The molecular reason for this is unclear; however, some peptides have common motifs that are particularly conducive to fitting into the MHC groove, and thus have "high promiscuity" when it comes to presentation by MHC [24].

Taking into consideration that most blood group antigens consist of a single amino acid difference between donor and recipient, and considering this information in the context of both known MHC restriction for such antigens and differences in promiscuity of such antigens, an elegant model emerges where the immunogenicity of a given blood group antigens is a direct function of the ability of a peptide containing the variant amino acid to fit into the presentation groove of the MHC. This is likely not only important in understanding the underlying biology of red blood cell alloimmunization, but may also be a useful predictive clinical tool in special patient populations who are particularly susceptible to sequelae of multiple alloimmunization. For example, in some centers, patients with sickle cell disease are given only red blood cells matched for RhD, Kell, Kidd, and Duffy antigens. This significantly limits the supply of red blood cells they can receive. However, a given sickle cell patient may have an HLA type known to be associated with non-responsiveness to a given antigen. In such cases, if blood were difficult to obtain, such analysis would provide a rational basis for which antigenic barrier to cross first in identifying red blood cell units. In cases where enough data are accumulated showing non-responsiveness with a given HLA type, not matching for that antigen in patients of particular HLA types may even be

justified; however, substantial research will have to be carried out before it is clear if this is a viable option.

4. Genetic predisposition to alloimmunization after transfusion

Although traditional immunogenetics have focused mostly on how variation in MHC affects immune responses, it is now clear that many common polymorphisms in immunoregulatory genes exist in the human population. Such variant genes include cytokines, chemokines, surface receptors, and signaling pathways involved in immunology. Polymorphisms can exert their effects by altering the amino acid sequence of an involved protein. Alternatively, polymorphisms in regulatory regions can affect efficiency of transcription and/or translation, thus altering function by affecting steady state or inducible levels of protein expression.

With one exception, polymorphisms in immunoregulatory genes that are known to affect immunity in other settings have not been evaluated in regulating response to allogeneic red blood cell transfusion. One retrospective human study has implicated the *TRIM21* gene (an immunomodulatory protein involved in interferon signaling), in affecting the age of red blood cell alloimmunization of children with sickle cell disease [33]. This very intriguing study suggests regulation of alloimmunization by non-HLA variation in immunoregulatory genes. However, it was a small study with subtle results. Moreover, a murine model of red blood cell alloimmunization showed no difference in antibody response to transfusion between wild-type mice and mice with a targeted deletion of the *TRIM21* gene [34]. In aggregate, it is unclear if these contradictory data indicate that *TRIM21* polymorphisms have no effect upon human red blood cell alloimmunization or if this simply reflects difference between *TRIM21* immunobiology in humans and mice. Additional analysis of human populations with polymorphisms in *TRIM21* will be required to resolve this issue. Moreover, assessment of how the panoply of known immunoregulatory polymorphisms may affect red blood cell alloimmunization will likely constitute an important area of future research.

5. Non-genetic factors and the effect of environment

In recent decades it has become well appreciated that innate immune systems have profound effects upon the ultimate outcome(s) of adaptive immune responses. In particular, inflammatory insults promote immune responses, which might otherwise not occur in an uninfamed state. In this context, we reported (in a murine model) that inflammation of the recipient at time of transfusion can have a profound effect upon humoral red blood cell alloimmunization [35,36]. Subsequent retrospective human analysis reported that patients suffering a febrile reaction around the time of red blood cell transfusion were more likely to make an anti-red blood cell alloantibody response than those who were not febrile [37]. Together, these data suggest that recipient inflammation, and activation of the innate immune system, may determine whether a patient becomes alloimmunized to red blood cell transfusion. We have subsequently reported

(in a murine model) that recipients not given an inflammatory stimulus not only fail to make an antibody response to allogeneic red blood cell transfusion, but that mice exposed to certain red blood cell antigens in the absence of inflammation become actively tolerized; such tolerized mice fail to make an antibody response to a subsequent red blood cell transfusion (in the context of inflammation), which would otherwise induce an antibody responses in naive animals [38]. This also demonstrates that a critical factor, which occurs independently of MHC status, significantly impacts rate of alloimmunization. Together, these data provide several lines of evidence in support of a red blood cell alloimmunization paradigm that includes recipient inflammation as one such critical factor.

The above data seem to paint a fairly straightforward picture in which inflammation promotes humoral alloimmunization to red blood cell transfusion and lack of inflammation promotes a non-responsive tolerogenic state. However, a more critical analysis of the data raises several important caveats and complexities. First of all, the data from the retrospective human trial are consistent with fever (and thus inflammation) promoting alloimmunization; however, the data are equally consistent with patients who are systemically immunosuppressed being incapable of spiking a fever. Moreover, the inflammation story became much more complicated by the observation that different types of inflammation have different effects upon red blood cell alloimmunization [39]. Thus, animal studies suggest that it is not simply a function of “inflammation yes” or “inflammation no”; rather more sophisticated analysis of immune activation will need to be carried out to properly test this model in humans. As always, observational human studies (be they retrospective or prospective) will suffer from issues of correlation vs. causality, and special care must be taken to interpret the data cautiously (*post hoc ergo propter hoc*).

6. Interface with non-human sources of antigen

The immune system of any given individual is a dynamic and evolving entity driven by initial recombination, melded by thymic and peripheral tolerance to self-antigens, focused by exposure to infectious pathogens, influenced by co-existence with resident flora, and refined by tolerance to paternal alloantigens expressed in utero and xenoantigens (or in rare cases alloantigens) consumed orally. Even identical twins diverge genetically the moment they are born and their immune systems begin unique life narratives of interaction with all sorts of antigens and under all manner of environments. As explored above, these immune melding experiences will likely have only subtle effects upon the differences in immune reactions to viral or bacterial pathogens, which differ from self-antigens to such an extent that no manner of fine tuning will eliminate such responses. However, in the context of alloimmunization to human blood group antigens, the interface of human immune systems with external antigens may have a profound effect. Because the antigenic differences are often only a single amino acid, evolved or chance encounters with peptides from non-red blood cell sources may have a profound effect upon red blood cell alloimmunization.

The extent to which peptides homologous to blood group antigens are found in food has engendered little attention and likely merits further analysis. From the standpoint of antibody interactions, the likelihood for meaningful biology is low, as most consumed proteins are denatured by cooking and/or broken down by gastric processes. Although some native antigen can be found circulating intravenously when relatively large quantities of purified protein are consumed, the physiological meaning of this is unclear. However, in the context of the peptide epitopes seen by T cells (presented in the groove of MHC), the antigen is a denatured proteolytic fragment by definition, and can readily tolerize the T cell compartment through gastric pathways. Environmental exposure to such peptides in food and particular in foods containing large volumes of blood, have the potential to regulate the immune system. Again, as in the context of peptide presentation by MHC detailed above, just as presentation of the naturally occurring polymorphism will vary based upon HLA type, so will the ability of variant peptides to fit into a given MHC groove vary from person to person. Thus, immunogenetics may also control the susceptibility of a given person to immunoregulation through encounter with variant peptides.

Mimicry between microbes and blood group antigens has received some attention, and certainly is more historically grounded in transfusion medicine due to the fact that the presence of anti-A and anti-B in individuals missing these antigens is due to immunization to similar (or identical) carbohydrate structures on gut flora. However, carbohydrate antigens are a different immunological entity with distinct biology from the vast majority of human blood group antigens. In the context of protein antigens, it has been observed that some microbes do in fact express surface epitopes that cross-react with antibodies specific for human blood group antigens [40,41].

Mapping crossreactivity between environmental microbes and blood group antigens at the T cell level is less straightforward, because unlike the relative simplicity of antibody binding assays, peptides have to be processed and presented in the context of a patient's MHC and incubated with a patient's T cells. However, we have recently reported significant homology between certain blood group antigens and peptides expressed by *Haemophilus influenzae*, *Bacteroides fragilis*, *Salmonella enterica*, *Lactobacillus vaginalis*, etc.) [42]. Moreover, in a murine model system, we have recently reported that antecedent exposure to a microbe expressing a peptide from a model blood group antigen results in a profound enhancement of primary humoral alloimmunization upon subsequent transfusion [42]. Unlike mimicry at the native antigen level, which would result in a patient with a positive serology despite no history of transfusion, priming at the T cell level is not detectable by clinical immunohematology as no antibody response is induced. Rather, the system is primed for a profound primary antibody response upon subsequent transfusion; thus, this constitutes a theoretical environmental event with the potential to have profound effects upon responder/non-responder outcomes in red blood cell transfusion. Of particular note is that based upon recent genomic mining of gut flora, it seems likely that many (if not the majority) of microbes that co-exist with human biology have not been identified; accordingly, expanding knowledge of the microbiome

will be of great benefit in further analysis and understanding of microbe/host effects upon transfusion based alloimmunization.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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