

Immigration control in the vertebrate body with special reference to chimerism

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The phenomenon of chimerism is reviewed against an understanding of adaptive immunity in vertebrates. It is shown that chimerism can be regarded as a ubiquitous condition, and this suggests that monophylysis has played little part in evolution. It is suggested that the adaptive immune response has a special role in facilitating the development of chimerism and that the consensus view of adaptive immunity as a rejection mechanism should be revised.

Introduction

The idea of a chimera was known to the ancient Greeks who saw it as an animal with the tail of a serpent, the head of a lion and the body of a goat. By meiosis the word came to mean “a vain and foolish fancy.” As Rinkevich points out,¹ in recent years natural chimerism, in the commonly accepted sense of an organism containing cells from two different zygotes, has come to be widely documented in at least ten phyla of plants, protists, invertebrates and vertebrates including mammals, which are the prime interest here. As a ubiquitous phenomenon is it far from a vain and foolish fancy, albeit the original Greek concept is likely to remain unreal.

In vertebrates the phenomenon of chimerism creates problems for the immunologists. All vertebrates have an adaptive immune response and in the conventional mind this equips them, as the name suggests, with the capacity to respond to foreign agencies in a rejectional manner tailored to be appropriate to what could be a hazard. Vertebrates under normal circumstances should not show chimerism

but, as will be seen, the principle concerned is not well founded.

In relation to the adaptive immune response which, in addition to chimerism, will be a main theme of this paper, the finding of Ray Owen² that in dizygotic twin cattle exchange of cells during fetal life led to a permanent state of hematopoietic chimerism is important. Medawar and Burnet deduced from Owen's finding that the rejection of foreign tissue that Medawar, in his ground breaking study of skin homografting,³ had found, need not take place if the foreign tissue concerned was encountered early in development. Medawar and his colleagues went on to show the following:⁴

If living cells from a mouse of the CBA strain are injected into an adult mouse of the strain A, the CBA cells will be destroyed by an immunological process, and the A-line mouse that received them will destroy any later graft of the same origin with the speed to be expected of an animal immunologically forearmed. But if the CBA cells are injected into a fetal or newborn A-line mouse, they are accepted; more than that, the A-line mouse, when it grows up, will accept any later graft from a CBA donor as if it were its own.

This statement, based on experiments whose design was deduced from Owen's study of chimerism in cattle, established the basis of Immunological Tolerance. Medawar, in his Nobel Prize acceptance lecture, observed that his experiments could be thought of as “an artificial reproduction of an astonishing natural curiosity.” Discussing his experiments, Medawar

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wrote “it does seem indeed that the antigens [of the tolerizing foreign tissue] must continue to be present, even though in quantities below the threshold of direct estimation, if a fully non-reactive [i.e. tolerant] state is to be maintained.” Medawar did not speculate on the nature of the persistent antigen and at that time the technology for tracking somatic cells was not as advanced as it now is. From studies of microchimerism⁵ it seems likely that at least some of the tolerizing cells did persist in Medawar’s mice.

While the studies of Medawar and his colleagues showed that rejection of foreign tissue did not take place if it was encountered early enough in life, the phenomenon of microchimerism in mammals involves not only transfer to and acceptance of maternal cells by the fetus, a finding commensurate with standard theories of immunological tolerance, but also transfer and persistence of fetal cells to the mother who is a fully developed adult in which immunological tolerance would not be expected. The unexpectedness of this finding of fetal to mother chimerism, an apparent exception to the rules of transplantation, is one of the main starting points for the present paper.

Exercise of adaptive immunity, it will be argued, often leads both to chimerism and maintenance of the chimeric state. It is suggested that much of contemporary immunological thinking is based on the study of artifacts which have distorted our understanding of the biological significance of the adaptive immune response. Not only is transplantation of organs an artifact with, except pregnancy, few natural homologs, but much of the remaining plethora of immunological work was created using reductional experimentation rather than by study of the response to invading exponentially growing living foreign organisms and immunogenic material derived from food.

It is proposed that polyphyletic chimerism is the usual condition for living organisms and that as Rinkevich¹ states “chimaerism challenges the traditional evolutionary dogma for the dominance of genetically homogeneous entities in Nature.”

Evidence for these propositions will be presented from: (1) Studies of adaptive

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immunity, initially concentrating on the standard displays of the phenomenon. (2) Widening of the approach to defense against infectious agencies. (3) Review of the non-specific innate immunity defense mechanisms to place the adaptive immune response in perspective. (4) A brief review of symbiosis to illustrate that cooperation, rather than conflict, has been a main driving factor in evolution and that this is relevant to the phenomenon of chimerism and the outcomes of exercise of adaptive immunity.

A final section will attempt to reconcile the various strands of approach and to present an overall view of chimerism and its relationship, particularly in vertebrates, with adaptive immunity.

The Adaptive Immune Response

Phylogeny. Adaptive immunity is seen only in vertebrates. Within that subphylum there are recognized many differences in both the outcomes of adaptive immunity that are taken to indicate an evolutionary sequence, from a simple condition in fishes, to more complex in mammals. It is not immediately obvious why vertebrates need more robust immunological defense mechanisms than do say, earthworms, which live in an enormous and varied collection of microorganisms. Nor is it apparent why mammals should need a more elaborate adaptive immune response than do say, cartilaginous fish. Adaptive immunity is supplementary to innate immune mechanisms, which are found in all living organisms. It should be stated here that it is not obvious within the current paradigm, that immunity is defensive against foreign and potentially dangerous invaders, why both adaptive and innate immunity are necessary.

Mechanism. The adaptive immune response provides, either by manufacture of antibodies and/or cytotoxic cells, a specific reaction to identify, and on the face of it, cast out foreign entities. Much

work has been done to reinforce the belief that T- and B-lymphocytes⁶ and dendritic cells,⁷ the main instruments of adaptive immunity, in cooperation with various agents of the innate immune response, are responsible for these rejection processes. The rejections are supposedly sustained by the development of specific immunological memory which allows a more rapid and effective response to second contact with the same foreign bodies, ostensibly in the absence of the initiating immunogenic stimulus: hence the use of the word memory by reference to mental capacity.

Transplantation. Adaptive immunity and one of what was then recognized as one of its main components, cellular immunity, became more important in the late 1950s when, partly to repair war damaged skin, the possibilities of transplantation of tissues and organs were beginning seriously to be considered.

Medawar and his colleagues in their work on rejection of skin grafts³ laid the foundations for the practice of transplantation, which can be seen as the creation of iatrogenic chimerism. Although with various false starts, as the hazards along the road were discovered, it is now commonplace in human medicine to transplant kidneys, heart, lungs, liver and bone marrow. All such organ transfers can readily be achieved between identical twins but transfer between non-identical individuals usually requires the use of agents designed to suppress rejection of the transplanted organs mediated by the adaptive immune response. In some instances, when the transferred cells include immunologically active lymphocytes, steps have to be taken to suppress potentially dangerous graft vs. host reactions.

Dineen and Szenberg,⁸ however, showed that that when skin allografts were apparently rejected, a portion of the donor tissue remained within the repaired dermis of the host organism. Though the graft had largely been destroyed, the residue meant that a state of chimerism was

created by the grafting technique. It cannot be excluded that this residue of tissue is responsible for maintaining both a low level response to the foreign tissue remnant and, by doing so, ensuring a rapid response to further challenges by the same organism, without rejection of the residual portion of the first graft. It is not recorded whether a second more quickly rejected graft leaves a residue. Similar examples of retention of foreign tissue in conjunction with an active adaptive immune response will be considered below in relation to infection and adaptive immunity.

In relation to organ transplantation, the question of whether cells from the graft persist after rejection of the organ as a whole seems not to have been determined. What is clear from an early paper by Starzl et al.⁹ is that, from large whole organ grafts, cells migrate out and become integrated parts of the recipient. In addition, recipient cells migrate into and become functional parts of the graft. Thus organ transplant recipients become complex chimeras and it is argued that the development of this state is a favorable indication for long-term graft acceptance, in which there can be relaxation of the toxic immunosuppressive protocols.

Much effort has been expended to understand the constraints on transplantation between genetically different individuals. It is widely accepted that so-called histocompatibility antigens on the surfaces of cells are the recognition factors from which rejection of foreign tissues by the instruments of the adaptive immune response is begun. These same molecules have come to be accepted, in addition to their recognition roles, as active factors in helping to drive immune responses. It is argued, particularly by J.L. Nelson, that the phenomenon of microchimerism is affected by histocompatibility differences between mother and fetus, but the exact nature and extent of this effect remains to be discovered.¹⁰

Pregnancy. Pregnancy in eutherian mammals involves temporary implantation of an allograft, which, it is widely supposed, the placental interface spares from rejection by the adaptive immune response. There are various credible ways in which this protection is thought to be achieved.¹¹ It is supposed that termination

of pregnancy at full term is not an immunological expulsion and, with few exceptions, the implantation of one fetus expressing paternal foreign antigens seems not usually to sensitize the mother to subsequent pregnancies with the same father. That an adaptive immune response is not a prerequisite of pregnancy is evident from the fact that scid mice lacking both T- and B-cells are not completely infertile. Whether pregnancy is enhanced or otherwise by the adaptive immune response seems uncertain.

It could be argued, that cognate with the findings of Dineen and Szenberg,⁸ pregnancy leaves a residue in the form of microchimerism, as does a skin allograft, and the role of the adaptive immune response is, perhaps in both instances, that of establishing and maintaining a stable, albeit low grade, chimeric state where there is an active awareness of introduced foreign tissue and the immune response is so tailored as to accommodate not reject. This interpretation will be further explored by looking at a range of communicable diseases.

Infectious Disease and Chimerism

The American Public Health Association has, for many years, produced a manual entitled "Control of Communicable Diseases." This useful document, now in its 19th edition, reviews the majority of the infectious diseases that are known in humans. The book is a compilation of expert views presented in alphabetic order of disease with specific disease sections presented in a standard format. It is intended primarily as an overall information source for workers in the field of preventive medicine. It makes somewhat uncomfortable reading for many experimental immunologists unused to thinking about living organisms as target immunogens.

Review of the manual reveals that there are a few hundred communicable diseases known in humans, more than half of which are viral. The uncertainty about the number is in part due to the fact that a number of named diseases have local or regional variants caused by organisms other than the type specimen. It also has to be pointed out that there are many gaps in our knowledge about the named diseases;

we know a little about the 25% of diseases with big impact but very little about the remaining 75%—most of which are of little commercial importance and, perhaps for this reason, little explored.

There are a number of general points that emerge from the manual that are relevant to the present discussion:

- In humans there are far more species of foreign organisms, mainly bacteria, regularly found as part of the normal buccal, gut and skin flora than are normally pathogenic. The point to be made is that foreign organisms per se seem only relatively rarely to pose a threat of pathogenesis. Whether there is in fact an immune response to each of the hundreds of species of foreign organisms that constitute the normal gut and buccal flora seems not to be clear. It should also be pointed out that the overwhelming majority of the many millions of species of microorganisms which are known to exist, but which are not represented in the gut flora, seem not in any way to impinge on the human species, not necessarily because the adaptive or innate immune responses will destroy them but, more simply, because there are no biochemical affinities between the species concerned that would be required to initiate invasion or sustain co-habitation.

- With the exception of a number of fungi and some protista, susceptibility to infection is universal, although with many diseases the age(s) of heightened susceptibility is/are often definable. Care must be taken to distinguish between susceptibility to infection, which implies that there are no absolute barriers to entry of the organism concerned, and susceptibility to disease following entry of the pathogen. If susceptibility to infection was a significant evolutionary disadvantage it is surprising to find it is almost ubiquitous.

- Many diseases arise as a secondary consequence of acquired immunodeficiency. It often appears that the causal organism was living unknown with the host until the immunodeficiency arose, disturbing what was previously a stable equilibrium in which the adaptive immune response, probably working in concert (or even in conflict) with the innate immune response, is the peace-keeping force. For example, in relation to what is regarded as one of the most

dangerous of all infectious conditions, only 10% of those invaded by the causal organism, *Mycobacterium tuberculosis*, develop the disease. The usual situation is that there is an unapparent infection with persistence of the pathogen and resistance to further infection. It is a paradox that we know too little about unapparent infections or the circumstances in which they arise in one healthy individual but not another. Evidence-based medicine in this instance seems not to have been sought. What should be emphasized is that it can be the asymptomatic persistence of the potentially pathogenic invader, not its rejection, which could be the usual part of the resistance to further infection. The role of the adaptive immune response in such common circumstances, if any, is probably in reducing the initial parasite burden and thereafter maintaining an appropriate and essentially symbiotic relationship. The advantage to the infectious agent, required to fulfill the definition of symbiosis as mutually advantageous, is that it has a stable home and nutrition. The potential advantages to the host are three-fold: (1) resistance to further similar infection, (2) creation of a state of activity of the lymphoid system which is advantageous in relation to resistance to other non-specific infections and (3) more speculatively, the invader brought in genes which the host can use either as a consequence of their expression in the invading organism or by horizontal gene transfer, of which more later. Although susceptibility to infection is almost universal, disease as a consequence of infection is far less common; the majority of organisms capable of causing disease, having gained access to the human body, persist there, but the hosts commonly remain symptom free, ostensibly immune and with unapparent infections.

- Many diseases are more severe and prolonged in their acute phases in immuno-deficient or otherwise stressed individuals who have reduced capacity for making adaptive immune responses. Clearly, the adaptive immune response

can be deduced to have a role in management of invasion.

- Chronic infection is the usual mode of host/parasite[†] interaction, often with resistance to further infection. In other words, vaccination, dependent on an active adaptive immune response leading to stable and indeed beneficial persistence of a potential pathogen, is a common natural phenomenon, particularly in relation to infective viruses. In those instances in which the parasite is “cleared” from the host there is commonly no residual immunity detectable on further challenge (e.g., with Borelia, Listeria and Chlamydia following chemotherapeutic eradication of the invading organism). It does seem in these circumstances that active adaptive immunity is often mediated by continuation of response to persistent stimulating antigen rather than requiring the evocation of a memory capacity in the absence of the initiating antigen. How common such circumstances are is not well documented, nor, as they are outside the contemporary paradigm for adaptive immunity, do they seem deliberately to have been sought. The possibility that the gut flora provides a library of cross-reactive epitopes is considered below.

One example from reductionist experimentation¹² illustrates, in relation to a communicable disease, one of the main thrusts of this exploration of the adaptive immune response. *Trypanosoma musculi* is, as its name implies, a parasite of mice. As few as one living organism put into a normal mouse will produce in 10 to 15 days a detectable parasitaemia which lasts for 12 days or so and then declines to zero. During this time it is possible to measure increases in specific anti-trypanosomal antibodies. Once the parasitaemia has declined further, challenge with the same parasite does not engender a further parasitaemia. At no time do the normal mice appear ill. If the experiment is repeated with T-cell deficient mice over three months or so, the parasitaemia becomes fulminant and eventually the mice die. Clearly *T. musculi* is a potential pathogen and the adaptive immune response involving T-cells is instrumental in controlling it. Further, in normal mice there is a memory of the response to the contact with the pathogen. An experiment

such as this is commensurate with what might be termed the standard interpretation of adaptive immunity as an effective means of both controlling and rejecting the parasite and safeguarding against second contact with the same organism by creation of a specific immunological memory. Immunologists can sleep calmly in their beds with such an experiment accomplished.

Except, when the previously infected and hyperimmune normal mice, which had substantial numbers of germinal centers in their spleens indicative of an active production of humoral antibodies, were dissected and kidney pieces implanted in uninfected normal mice, parasitaemia followed. Backtracking, the parasites were found to be in special loops of the blood system of the hyperimmune animals, presumably bathed in antibody. Perhaps they even used the anti-trypanosomal antibody as a source of nutrition as it is manufactured to be specific for them. Attempts to bring the parasites in the hyperimmune mice back into the main blood stream by thymectomy, irradiation and bone marrow injection or by injection of massive amounts of steroids both failed. Pregnancy, however, succeeded and the pregnant mothers quickly became parasitaemic—with no obvious adverse consequences it should be stressed. It is not recorded whether the offspring were infected but it is difficult to see why they should not have been.

Communicable diseases, in many instances, have the same, largely asymptomatic, persistence of introduced foreign organisms, which constitutes chimerism, as do natural transfers of conspecific cells seen in the fetal/maternal microchimerism; despite, or perhaps because of, the adaptive immune response there is persistence of the stimulating foreign entity. Both the overall view of infection in humans and the experimental studies on *T. musculi* bear out this view.

It must also be noted that host parasite interactions are mutual not only one way. The host can adapt by means of the adaptive immune response which offers a wide variety of idio- and allo-types of antibody—the spectrum and quantities of which can vary with increasing time after the initial contact with the parasite

[†]In this paper parasite will be used to indicate an organism, metazoan, fungal, bacterial or viral with the potential to harm its host.

immunogens. More importantly, the parasites are far more capable of adjusting to adverse circumstances by selection of germ line genetic variants than are the host organisms. The replication of parasitic microorganisms is usually very rapid, creating the conditions from which selection can occur of variants better able to ensure preservation of the parasitic lineage.

Variation of parasite genetic constitution, consequent upon a high mutability rate, and variation of expression of parasite antigenicity, as for example in trypanosomes growing in a highly susceptible host, *T. rhodesiense*, in the domestic humped cattle in East Africa,¹³ are well-known phenomena. Such things are the bane of the lives of vaccine manufacturers and usually interpreted as enabling parasites to evade the adaptive immune response of the host organism. It can equally be interpreted as the parasite endeavoring to find a phenotype that the host organism can accommodate. If the host dies as a consequence of parasitic infestation, so does the parasite. If the parasite is rejected, that is the end of the line for the parasite. It seems common sense that mutual accommodation with peaceful coexistence should be the preferred strategy, and it does seem, by looking at the large picture in the American Public Health Association manual, that this is a common occurrence.

From a wider perspective, Dawkins¹⁴ draws attention to a number of instances in which parasites have the capability of manipulating the physiology of the hosts to the advantage of the parasite. Mercifully these examples are relatively rare and seem not to detract from the present argument that both host and parasite have a vested interest, in normal circumstances, in creating an interaction that allows both to survive.

If, as is argued, operation of adaptive immune mechanisms can fail totally to reject potentially hostile foreign agencies, but nevertheless can result in effective rejection of iatrogenic transplants, there is the question of why this is so. Matzinger has offered an answer to this question.¹⁵

Matzinger proposed that immune responses are triggered in some way by what she argues is damage, such as would be manifest if cell death occurred. Such

a notion is readily demonstrable by comparing two possible outcomes of injecting a mouse subcutaneously with sheep red blood cells. If the injection is done carefully with a small volume of fluid, no antibody production follows; if the injection site is pinched with the fingers of the injector, then substantial humoral antibody production is as normal.⁸ The use of powerful stimulants of the innate immune system, such as Freund's complete adjuvant, to enhance immune responses illustrates the same effect, albeit with a greater degree of tissue damage due to the non-biodegradable component of the adjuvant. Transplantation of an organ such as kidney, heart or liver involves much cutting and, of necessity, considerable ischemic tissue damage. According to the notions of Matzinger, the damage initiates the rejection reaction that will lead to loss of function of the graft unless powerful immunosuppressive agents are deployed. Possibly, steps taken to minimize the damage and/or reduce the consequences of the damage consequent upon the creation of states of iatrogenic chimerism would perhaps be better means of facilitation of graft acceptance than heavy and potentially damaging application of powerful immunosuppressants. If Matzinger is right, the current methods of organ transplantation involve the creation of "adjuvant" damage, which facilitates identification and gross reduction of the introduced tissue. It could be that in some instances long-term graft persistence, associated with progressive reduction of immunosuppressive measures, illustrates what can happen once the period of initial damage has passed.

Innate Immunity

It is germane briefly to review innate immunity better to put the adaptive immune response in perspective of some of the other various mechanisms that operate at the interface between living organisms.

Innate immunity in various forms is present in all living organisms. In its basic condition it serves to identify other living organisms, some of which could represent a conflict of interest in that they could be predatory and should be avoided or

attacked defensively. By the same token, in some circumstances, foreign organisms could be a source of nutrition and therefore actively sought. Some of the molecular messengers that facilitate these recognition processes are to be found in nearly all multicellular organisms¹⁶ and there are similar molecules in protists and prokaryotes.⁸

The innate immune response is considered to depend upon the existence of families of ubiquitous receptors, Toll-like receptors (TLR) for example, which can bind ligands on foreign entities. The ligand/receptor binding initiates a signaling system with a wide variety of consequences, of which phagocytosis and destruction of ingested material is a major aspect. In contrast to adaptive immunity, innate immunity is not usually sustained or considered to have a specific memory component. Broadly, the innate immune response creates, in various guises, inflammation to which the instruments of the adaptive immune response can also contribute. It should be noted, that while there are many and complex deficiencies of the adaptive immune response, some congenital, some acquired and some of which are of relatively low morbidity, major failure of innate immune responses is usually quickly lethal.

In all triploblasts, TLRs are associated, inter alia, with phagocytic cells, a principle component of innate immunity, which play a role in defending the body against invasion by potentially harmful microorganisms. Prior to deployment of these internal instruments of defense, initial protection against invasion usually includes the physical barriers offered by the skin and, in vertebrates, mucous epithelia. Within the mucus are found a variety of anti-microbial agents. Entry to the body by means of the buccal cavity and the

⁸A.J.S. Davies, unpublished lab experience. Try it.

¹⁶TLRs are present in vertebrates, as well as in invertebrates. Molecular building blocks of the TLRs are represented in bacteria and in plants, and plant pattern recognition receptors are well known to be required for host defense against infection. The TLRs thus appear to be one of the most ancient, conserved components of the immune system. Wikipedia, January 4, 2012.

esophagus is restricted within the stomach where, under normal circumstances, the acid pH kills most ingested microorganisms. It also has to be noted that the skin and the gut both have complex micro-organismal floras, microbiomes, which, by occupying ecological niches, can effectively exclude both invasion by non-indigenous organisms and overgrowth by potentially pathogenic components of the normal flora.¹⁷ The recent popularity of fecal bacteriotherapy in humans illustrates how practically the engineering of the gut flora can benefit patients. The veterinary profession has, for a number of years, used similar methods deploying cecotrope feeding in rabbits with intestinal problems attributed to imbalance in the gut flora.

Protection against invasion is thus a complex multi-layered phenomenon of which the internal innate immune mechanisms operate quickly in identifying, capturing and destroying the bulk of the invaders that have gained access to the body. Without this system functioning adequately, death as a consequence of overgrowth, particularly of bacteria, is usually swift. Innate immunity is seen in all triploblasts but only in the vertebrates is there the additional, relatively slow and chronically reacting, system of adaptive immunity. It is pertinent to ask what this system affords as an internal defense mechanism against attack by other living organisms?

Perhaps a clue arises from a short review of symbiosis, which, in many instances, can be regarded as examples of chimerism.

Symbiosis

Symbiosis from the earliest forms of life to the present day is a ubiquitous phenomenon. The most striking examples involve endosymbiosis, which occurred following initial acts of cell fusion. These are deep and increasingly well researched issues, recently summarized by Yutin et al.,¹⁸ that point to the origin of the eukaryotes from archaean and bacterial ancestry and which present the genetic evidence that some of the organelles in eukaryotes, particularly chloroplasts and mitochondria, still show by their genetic content their ancestral origins. In addition, it is clear that horizontal gene transfer from the various

endosymbiotic elements took place so that the present nuclear material of eukaryotic cells contains archean and bacterial genes as integrated and vital elements of their constitution. The current (January 7, 2012) Wikipedia entry for Endosymbiotic Theory summarizes well the array of evidence and the historical development of the topic from 1883¹⁹ when it was first suggested, incidentally, that green plants could have been derived from the union of two organisms.

The question of conflict of interest, which is such an important part of darwinian thinking, leading to evolution by survival of the fittest must surely have existed in these early days in the development of life forms. The horizontal gene transfers that are postulated to have occurred and the genetic simplification of, for example, mitochondria which contain only a small, but important, part of their original gene constitution suggests that, in whatever time it took, some organisms which entered into endosymbiotic associations lost their independence and essentially became slaves. It is important nevertheless to emphasize that, despite potential conflicts of interest, cooperation between very disparate living organisms was an integral part of the early steps in the evolution of living organisms.

Symbiosis in more simple forms affects all present day living organisms and ranges from the mycorrhizal associations of the great majority of rooted plants, through the extraordinary alliance of the animal part of corals and their contained algae. It includes the lichens, almost the dominant plant vegetation in the world with their obligate mutualistic partnership between the fungal thallus and the photosynthetic algae. The symbiotic bacterial flora of tribloblasts offers a variety of scenarios, including the siboglinid tube worms, which are to be found in the deep ocean adjacent to volcanic vents; they have no mouth and no gut but derive all their nutrition from endosymbiotic, sulfur metabolizing bacteria. Herbivorous ungulate mammals cannot properly digest cellulose without a gut flora, neither have they any source of vitamin B12 aside from the gut flora. Only recently is it becoming apparent that the gut flora of the species *Homo sapiens* is probably involved in

influencing a wide variety of aspects of the human condition, and it is quite likely that specific intervention accurately targeted to the gut flora will be a significant component of a variety of medical treatments over the next decade.²⁰

Dawkins,¹⁴ in his prophetic paper discussing parasites and what he terms the paradox of the organism, argues that it is the genetic material which is subject to darwinian natural selection rather than individual organisms. He states: "an individual organism is an entity all of whose genes share the same stochastic expectations of the distant future."

Although in his paper Dawkins did not intend to include mitochondrial genetic material, nor that of the gut and skin flora, the argument that he advances clearly holds for these epigenetic symbionts. The current definition of chimerism involves eukaryotic organisms containing cells derived from different eukaryotic zygotes. It could credibly be argued that this definition should be extended to include the endosymbiotic states that led to the evolution of eukaryotic cells and the ubiquitous associations of bacteria and viruses with eukaryotes. It is simply not possible on the basis of our contemporary view of the diversity of living organisms and the ubiquity of a wide variety of chimeric states to avoid the realization that monophyletic is vanishingly rare, if indeed it exists among present day living organisms. The interactive systems that are consequential upon a polyphyletic way of life are little understood but, it is contended here, they involve in part in vertebrates the adaptive immune mechanisms.

Reconciliation

It is argued here that chimerism is a common natural phenomenon and that it can be given a wider definition than has so far been the case. Microchimerism in particular, quite aside from the light it may throw on our understanding of stem cells and their role in tissue regeneration, can be argued to be cognate with a far wider range of interactions with living organisms. One mechanism in vertebrates that is instrumental in facilitating such interactions, it is proposed, is the adaptive immune response. This phenomenon, a late entrant

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to the array of evolved physiological phenomena, seems to present better emphasis on cooperation rather than the conflict of interest which can seem intrinsic to the darwinian mode of evolution insofar as it involves environmental interactions with other living organisms. As Matzinger puts it, “Life did not take over the globe by combat, but by networking.”²¹

Humans are a very successful and competitive species, as testified by their present dominance of the globe and its opportunities for development. Whether this control will persist for another 200,000 years (the time it is reputed *Homo sapiens* has been on the march from his starting point in the East African hinterland) remains to be seen, and speculation on this issue is outside the present remit. Present interpretation of the adaptive immune response as primarily a rejective defense mechanism relies heavily on the medical ethos in the Western world, which sees infectious disease and its remediation as a major war that, for a time being, was thought to be won, partly by implementation of a variety of public health measures and, more recently, by the indiscriminate use of manufactured antibiotics. The argument in this paper is that from an evolutionary point of view infectious disease can better be seen as two sets of organisms seeking rapprochement in which the adaptive immune response has a critical role and where a state of symbiotic chimerism is the preferred outcome.

Thinking about the importance of the adaptive immune response as an interface mechanism in relation to the evolution of humans and vertebrate ancestors draws away from a predominantly anthropocentric view. What is important is to look carefully at the adaptive immune response and see that clearly it can be interpreted as defensive, but that its most potent effect

could be to act as an immigration control mechanism for potential parasites, not automatically to reject them.

The bulk of the symptoms and the consequent morbidity of disease, particularly communicable diseases, derive from activity of the immunological apparatus, both innate and adaptive. If the immune responses, particularly the adaptive response, is to be protective it is extraordinary that immunopathology is responsible for almost all of the problems that can arise consequent upon invasion by pathogens capable of leading to disease.

A classic illustration of immunopathology accrues from infections with *Theileria parva* in the non-indigenous farmed cattle in East Africa. This protist organism gains entry to its mammalian hosts via the mouth parts of brown ear ticks. In the host it invades and transforms a wide variety of lymphoid cells, leading to release of massive amounts of cytokines, causing drastic alterations in vascular permeability²² that quickly have lethal consequences on the host animal. It must be recorded that indigenous species of *Bos*, water buffalo and antelopes such as water buck often carry the parasite apparently asymptotically, as they doubtless have for many millennia before the humped cattle were imported into East Africa from regions where there were none of the vectorial ticks for species of *Theileria*.

The adaptive immune response has both advantages and disadvantages, though the former presumably far outweigh the latter, otherwise the existence of the mechanism, its ubiquity in the vertebrates and its increased complexity and sophistication coming up the evolutionary tree from primitive vertebrates to primates, would not be so evident. It is a remarkable process, which, on the

evidence presented, does not seem to be exclusively, if at all, a rejection mechanism. In those circumstances in which it appears a parasite has been lost but the adaptive immune response is still going strong, it can be argued that the gut flora, which has perhaps one hundred times more disparate genes and their products than has, say, the human genome, provides an adequate library of cross reactive epitopes that can maintain an active immune response that was initiated by an externally derived antigenic challenge. Reinforce this with the enormous battery of potentially immunogenic macromolecules getting into the blood stream from dietary sources, and it needs to be questioned whether immunological memory and the battery of “memory” cells which is associated with contemporary immunological thinking could better be regarded as manifestations of a low level ongoing accommodating immune response.

One of the main talking points in relation to adaptive immunity has been the issue of how vertebrates avoid problems of self-recognition with adverse consequences. The existence of autoimmune diseases seems to illustrate what problems can arise if self-recognition is imperfect. The argument usually advanced²³ is that in the early stages of development, lymphocytes on the T-cell pathway in the thymus that are capable of reacting against self are eliminated. If this is correct, Immunological Tolerance as put forward by Medawar and his colleagues, arising as a consequence of neonatal injection of foreign cells, is associated with elimination of those host cells capable of reacting against the intruders which will now be regarded as self. Such a hypothesis might be termed passive in that it depends upon the removal of the relevant reactive cells. This ingenious explanation may prove correct, but the argument advanced in the present paper is that tolerance of foreignness under appropriate immunological control is, in fact, an active process. Recent studies by Faulk and his associates²⁴ suggest that autoimmunity, particularly insofar as it relates to humoral immunity, is controllable by active regulation of the efferent arms of the immune response rather than by, as was earlier thought, deletion of components of the afferent arm.

The human genome, now almost fully analyzed, has, as do other vertebrate genomes, substantial incursion of genes that derive from earlier endosymbiosis and/or later infection. Huebner and Todaro, in relation to included potentially oncogenic viruses,²⁵ pointed out these entities could well be responsible for enabling mitosis in the continuously replaced epithelia which characterize the vertebrates. Just how many viral sequences are present and active in the human/vertebrate genome is uncertain, but clearly the capacity of viruses to use the genetic apparatus of eukaryote cells, either as epigenetic entities or by direct incorporation into the genome, could enable evolutionary changes at a faster rate than by

accumulation of point mutations. The adaptive immune response as presented here has the attributes that enable it to effect accommodation of foreign entities, and as such could be seen as a facilitator of evolution.

Sagan and Margulis summarize the situation as follows:

We are just beginning to scratch the surface of the communication between our commensals and us. We are an environment to an uncountable number of symbiotic, commensal and pathogenic organisms, each of which has had evolutionary time to learn how to use and misuse our immune system. As we expand our picture of the immune system from an army of lymphocytes patrolling

*the body for foreigners to an integrated group of communicating tissues, all working to maintain tissue integrity and health, we will necessarily need to include the signals from the non-self organisms that take advantage of that health or that help maintain it.*²⁶

The phenomenon of chimerism and its importance are just being discovered. It will be an exciting time as the complexities of interaction between the living organisms are further discovered. The suggestion made some years ago that the adaptive immune response is designed not to reject, but to accommodate,²⁷ although counter intuitive, seems in the light of the recent studies of microchimerism, to be increasingly tenable.

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