



Microbes, molecular mimicry and molecules of mood and motivation



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ABSTRACT

The hypothesis proposed is that functional disorders, such as irritable bowel syndrome, chronic fatigue syndrome and anorexia nervosa are caused by auto-antibodies to neuronal proteins induced by molecular mimicry with microbial antigens. The age incidence of these conditions, the marked female excess, increase with economic and technological advance, precipitation by infection, and the paucity of histological changes are all consistent with the hypothesis. It can be tested directly using human sera to search for cross reaction with brain proteins in model systems such as *Drosophila melanogaster*. The conditions might be amenable to treatment using pooled immunoglobulin. Identification and elimination from the microbial flora of the bacteria that express the cross reacting antigens should be possible.

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Introduction

Functional disorders, such as irritable bowel syndrome (IBS) [1–4], chronic fatigue syndrome (CFS) [5–7], and anorexia nervosa (AN) [8–10] are a major cause of morbidity. They constitute a considerable proportion of the workload of the average general practitioner but the pathogenesis remains a mystery. Unlike most diseases that inflict mankind there are no consistent microscopic changes in body tissues to give a clue to their cause. These functional disorders, however, do have a number of features in common:

1. They are much more common in women than in men.
2. The onset of the disorder is most likely to occur in the middle years. The age incidence rises to a peak in the second, third or fourth decades, depending on the specific condition. The incidence then declines and onset in old age is uncommon.
3. There is some evidence that they are becoming more common with technological, social and economic progress in society.
4. There are suggestions that infection can precipitate and exacerbate the conditions but the links remain somewhat tenuous.
5. Diagnostic histological changes in tissues are absent.
6. Psychological factors might be important, but are unconvincing as the primary or major cause.

The hypothesis explored in this article is that these features can be explained by molecular mimicry between our microbial flora and neuronal proteins of the hypothalamus and gut; leading to auto-antibodies which affect mood and motivation.

The microbial flora

There are approximately ten times as many bacterial cells in the gut as there are human cells in the entire body. The number of different bacterial species that can be present is of the order of 400–800, with perhaps 40–80 present at any one time. Each bacterium has, on average, 3000–5000 genes coding for 3000–5000 proteins. The size and complexity of the bacterial proteome is therefore immense but it cannot be enumerated precisely because there is genetic overlap between different species and there is genetic diversity within a species [11,12].

The human genome has approximately 20,000 genes in the haploid set. The number of proteins, however, is in excess of 250,000. Human genes have several exons and differential splicing of the RNA transcripts of the exons can produce several different proteins. Furthermore human enzymes add and subtract chemical groups thereby increasing protein diversity [13].

The acquisition of immunity and the generation of specific antibody involve the coordinated action of macrophages, T lymphocytes and B lymphocytes. Macrophages phagocytose bacteria, kill them using intracellular enzymes, and then slice their proteins into polypeptides 8–12 amino acids long. The polypeptides are then posted on the surface of the macrophage in the groove of a surface protein termed MHC2 (major histocompatibility protein type 2). The task of the T and B lymphocytes, acting together, is

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to use the polypeptide as a code for the protein from which it was derived. If the protein is judged to be different than self and if it comes from a potentially pathogenic organism then antibody molecules which recognise the protein are generated. But if the protein is similar to self-proteins the generation of antibodies would be harmful. The precise mechanism is not fully understood but a key part is formation of a complex between the T cell surface receptor, the polypeptide and the MHC2 molecule. The recognition step is therefore dependent on the shape of the specific T cell receptor. There are both helper and suppressor T cells and in some way the helper T cell leads to antibody production by B cells whereas the T cell suppressor inhibits antibody production [14].

The task of the immune system is extremely complex and as argued previously this can be analysed in terms of statistical decision theory [15]. There are ten trillion possible ways in which 20 amino acids can combine to form a polypeptide chain ten amino acids long ($20^{10} = 10^{13}$). The total number of proteins in the combined human and bacterial proteome is probably less than a million, and with the average protein 300 amino acids long the total number of polypeptides of length 10 amino acids is less than 10^9 . Thus polypeptide chains of 7 amino acids ($20^7 = 1.28 \times 10^9$) are probably sufficient to code for every possible protein. The key receptor is the T cell receptor and the key decision is to differentiate similar to self from different than self.

A major problem for the decision process is that there is extensive overlap between bacterial genes and proteins and human genes and proteins. Protein structures which perform essential functions are preserved in evolution and we literally share out genome and proteome with the rest of creation. If a pathogen shares proteins with the human host then the task of generating antibodies to just the non-shared proteins is made more difficult. If the first exposure to the pathogen is in high dose and life threatening infection occurs then the decision must be swift and the risk of error will rise. Equally if first exposure is later in life when the immune system is deteriorating then again the risk of error will rise [15].

Gender differences

Decisions are made in uncertainty and even a highly sophisticated decision system operating an optimal decision strategy is prone to error; the more complex the system, the more redundancy that is built into the system, the lower the error rate will be. But errors cannot be eliminated completely. These are fundamental properties of statistical decision theory [15].

Let us define failure to respond to a foreign protein on a pathogen as a false negative decision. Conversely responding to a protein which is similar to self is a false positive decision. Adjusting decision criteria to reduce false negatives automatically increases false positives and *vice versa*. This again is a property of statistical decision theory. A false negative response will increase the risk of infection. A false positive will increase the risk of autoimmune disease. Some individuals will err on one side and some on the other. It is therefore noteworthy that men as a general rule are more prone to suffer from infection while women are at increased risk of autoimmune disease. This applies particularly to the organ specific autoimmune diseases in which auto-antibodies are thought to play a role, e.g., thyroid disease, pernicious anaemia, myasthenia gravis and primary biliary cirrhosis [16]. The female to male ratio in these conditions is of the order of 10. The female excess in IBS, CFS and AN is equally extreme [1–10] and therefore this fits with the idea that auto-antibodies to nerve cells could be part of the pathogenesis of these conditions.

Age incidence

Morbidity and mortality increase in incidence with age because the majority of diseases are a consequence of a gradual deterioration in the performance of our complex information processing systems. Disease caused by common pathogens on first exposure, however, generates a different age incidence curve. The probability of first exposure to common organisms falls exponentially from birth. The more common the organism the more rapid the exponential fall will be. But the probability of error on first exposure will rise as a power function of age because the immune system ages as do other bodily systems. Thus the age incidence curve we would predict for an autoimmune disease arising as a consequence of molecular mimicry is one that rises to a peak in middle life and then falls. Furthermore the more common the organism the earlier the peak will be, the less common the organism the later the peak will be [15].

This form of age incidence curve is seen with the functional disorders. AN peaks in the second decade, whilst IBS and CFS peak in the third and fourth decades. These conditions do not arise for the first time in old age, because by then there are virtually no new bacteria to meet.

Temporal changes

The spread of microbes is influenced by technological, social and economic change. A striking historical example is leprosy. Leprosy is a disease of poverty, overcrowding and poor hygiene; it is spread by prolonged personal contact. The disease was not uncommon in Europe in the middle ages but is now very rare. Another example, more directly relevant to this thesis, is poliomyelitis [17]. Epidemics of paralytic poliomyelitis arose amongst the better off in society at the end of the nineteenth century. In this case poverty, overcrowding and poor hygiene causes rapid spread of the virus and infants experiencing these conditions were exposed in the first year of life. Improved social conditions slow the rate of spread of the virus and in these circumstances first exposure occurs later in childhood. For reasons which are not fully understood later exposure led to an increased risk of paralysis.

A further example is glandular fever caused by the Epstein–Barr virus. The disease glandular fever is rare in African children and in the lower social classes in Europe and North America. These children are exposed to the virus early in life and have only a mild illness. Middle class children in Europe and North America can reach the teenage years without having been exposed to the virus. First exposure in the teenage years is more likely to lead to a severe disease [18].

There are probably several factors involved in increasing severity of disease with later exposure [12,15]:

1. Infants exposed in the first six months of life will have partial protection from maternal IgG and if breast fed from maternal breast milk IgA.
2. A decreased rate of circulation of organisms leads to a change from endemic to epidemic spread. Epidemic spread leads to later exposure and a higher dose on first exposure [12].
3. The Epstein–Barr virus is spread by bodily secretions and close contact in the teenage years could be associated with a larger dose on first exposure.
4. The immune system deteriorates with age, as do all body systems, so that errors are more likely to occur. This includes both false positives and false negatives. An impaired response is more likely to lead to severe infection as well as autoimmune disease [15].

A whole range of immune mediated diseases have increased in technologically advanced countries over the last 50 years. These include asthma, hay fever, eczema, type 1 diabetes mellitus and multiple sclerosis. The term “hygiene hypothesis” is often invoked. It implies that social advance leads to a change in the pattern of exposure to microbes and in turn this leads to an increased risk of immune mediated disease [19,20].

IBS, CFS and AN are also on the increase in technologically advanced nations [1–10]. Once again this fits with the concept of an auto-immune mechanism.

Infection

There is evidence that infection can precipitate or exacerbate functional disorders [21–24]. The onset of IBS commonly follows an episode of infectious diarrhoea. CFS can be triggered by infectious mononucleosis and viral hepatitis. More recently there is increasing interest in the concept of “cross talk” between the microbial flora and the enteric, autonomic and central nervous systems [23–25]. There are a number of ways in which bacterial secretory products could influence neuronal function but these operate in both men and women and at all ages. Only auto-antibody formation is predominantly female and arises in the middle years.

Histological changes absent

Infection normally causes damage to tissues and triggers inflammation. Bacteria and viruses can damage cells directly and they stimulate an immune response which also causes cellular damage and provokes inflammation. Indeed much of our understanding of disease and our classification systems of disease depend on the appearance of pathological changes assessed by the light microscope.

The presence of histological change is a clue to causation but an absence of histological change is not necessarily an absence of clues to causation. Molecules, such as bacterial toxins or auto-antibodies, can cause functional effects without histological change. There is increasing evidence that bacterial toxins can act on the neural systems controlling respiration to cause sudden infant death syndrome [26,27]. This results in death without diagnostic histological changes at autopsy. Another example, more directly relevant to the thesis in this article, is myasthenia gravis. An auto-antibody to the acetylcholine receptor protein causes muscular weakness. The disease is more common in women than in men and the peak incidence is around 30 years of age [28]. Auto-antibodies have been found in patients with anorexia nervosa recognising circulating regulatory peptides and neuronal proteins in the hypothalamus [29–32]. There are also reports of auto-antibodies to serotonin in CFS [33]. Post-streptococcal chorea is caused by auto-antibodies to basal ganglia proteins which cross react with the streptococcal surface antigens [34,35]. In all these cases there is an absence or a paucity of histological change, and certainly no diagnostic microscopic changes.

Auto-antibodies to enteric, autonomic or central nervous system neurons are a plausible explanation for functional disorders. The blood brain barrier will prevent access to much of the central nervous system but fenestrated capillaries occur in the hypothalamus allowing IgG antibodies to the site that controls the autonomic nervous response and emotional responses.

Psychological theories

The functional disorders are often termed “psychosomatic diseases”. This term has two meanings. In older literature the term was synonymous with psychogenic implying “all in the mind” or

imaginary disease. The more modern meaning, however, is that psychosomatic disorders are caused by the interaction of both psychological and physical factors.

It is the former idea that leads to unconvincing explanations. There might, for instance, be an increased incidence of physical and sexual abuse in childhood in those who go on to manifest functional disorders. It is easy to see how this could influence symptoms in adults but it stretches credulity to imagine abuse as the sole and sufficient cause of the functional disorder. Equally modern concepts of the perfect physical form promoted by the fashion industry will influence teenagers to diet but surely there must be something more profound and fundamental to induce emaciation and death by starvation or suicide.

Discussion

Auto-antibodies to neuronal proteins induced by exposure to microbial antigens are a plausible explanation for functional disorders such as IBS, CFS and AN. The age incidence, increased frequency in females, temporal changes in incidence, lack of histological changes and evidence of microbial precipitation all fit with an autoimmune condition. But this does not preclude a role for psychological factors. Auto-antibodies acting on the limbic system could induce extremes of emotion including disgust and fear. These then become linked, in the minds of adolescent girls, to culturally determined ideas of what is, and what is not, the ideal body shape and size. It is then a small step for disgust and fear to be directed to food and obesity which the fashion industry currently demonizes [10].

Auto-antibodies to regulatory peptides and to serotonin have been found in patients with AN and CFS [29–33]. Furthermore the concept of bacterial translocation and molecular mimicry leading to auto-antibody production has been raised in these publications. Thus there is already some direct experimental support for the hypothesis we propose.

To investigate this hypothesis we need to search for antibodies to neuronal proteins in patients with the above functional disorders. A key aspect of the hypothesis is that genes and proteins are conserved in evolution so that animals as diverse as flies, mice and men will have similar neuronal proteins. The detailed anatomy of the brains of flies, mice and men is clearly different but the building blocks are similar [36,37]. This means that we could use flies, such as *Drosophila melanogaster*, as a primary model organism to detect human auto-antibodies. *D. melanogaster* has, in fact, been used for over 100 years in many areas of research into human disease and function. In neuroscience, research using the vast array of genetic and molecular tools in the fly has been fundamental to our understanding of, for example, learning and memory, sleep, circadian rhythms and the neuroendocrine regulation of ageing. If human auto-antibodies bind to *D. melanogaster* neuronal proteins this can be visualised using immunofluorescence and confocal microscopy. The proteins can be subsequently identified and their sequence determined using the wide array of techniques available in this model organism.

If auto-antibodies have a significant role then immunotherapy using pooled IgG from healthy subjects is a treatment option in severe cases, particularly when life is threatened, as in AN [10]. The longer term aim would be to identify the specific cross reacting proteins carried by bacteria of the microbial flora, which induced the auto-antibodies in the first place. The bacteria of the microbial flora of an individual are only a subset of all bacteria and it should be possible to displace the offending organism and replace it with a closely related organism that does not display the causative protein. Furthermore we are increasingly of the view that only bacteria that invade the body are likely to provoke and maintain high

titre IgG auto-antibodies and therefore identifying the offending bacteria and replacing them might not be as difficult as appears at first sight [38,39].

Conflict of interest

The authors, Morris, Broughton and Wessels declare that they have no conflicts of interest with regard to this publication.

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