



Give us this day our daily germs

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Are we sparing the dirt and spoiling our children's immune systems? The theory that some germs are necessary in developing healthy immune systems is gaining credence as more evidence emerges. It is vital that we find out which germs are needed, when and how, before the increase in diseases attributable to faulty regulation of the immune system (allergies, autoimmunity, inflammatory bowel disease) spirals out of control.

The allergic disorders (asthma, hayfever, eczema) are much more common in the rich parts of the world than in the developing countries. There has been a steady increase over several decades so that allergic symptoms now afflict as many as 40% of all children in some European or American inner cities. Such a rapid increase cannot be due to genetic changes in the population, and must have an environmental cause (Figure 1).

Numerous groups have looked very closely at the epidemiology of allergies, and the major findings are shown in Table 1. These findings are all compatible with the view that the crucial factor is diminished exposure to microorganisms because of increased hygiene and antibiotic use. Such observations, coupled to increasing evidence that certain microbial components play a role in the correct maturation of the immune system, have led to the formulation of the 'hygiene hypothesis'. This attributes changes in disease patterns in rich countries to inappropriate 'education' of the immune system.

The immune system resembles the brain in that we are born with the hardware, and a limited amount of software and data, but correct function depends heavily on the information fed in after birth and on the sequence in which that information is received. This schedule of information should not deviate too far from that which our evolutionary history has set up the system to expect.

Epidemiology alone cannot prove this hypothesis, but it is now possible to identify organisms to which our exposure has unquestionably diminished. Some of these can be used to treat allergies in experimental models; the immunological pathways involved are starting to be identified, and human clinical trials are confirming the efficacy of this treatment. Thus the evidence is now extremely strong.

Back to bacterial basics

Which are the important organisms, and how do we know that exposure to them has decreased? A study of Italian army recruits demonstrated that exposure to childhood virus infections does not protect from allergies, which fits with the fact that children in inner cities are heavily exposed to these infections and still have a high and rising risk of allergies. Diminishing contact with bacteria as a cause for allergies fits the epidemiology much better. Very detailed studies of farming communities in southern Germany, where the barn containing the cows communicates directly with the house, revealed that more time spent in the barn as a young child and higher content of bacterial products in bedding resulted in fewer allergies (Riedler *et al.*, 2001).

This is good evidence that bacteria are involved, but does not identify a particular genus of organism. However, we know that mammals evolved in mud, and humans then

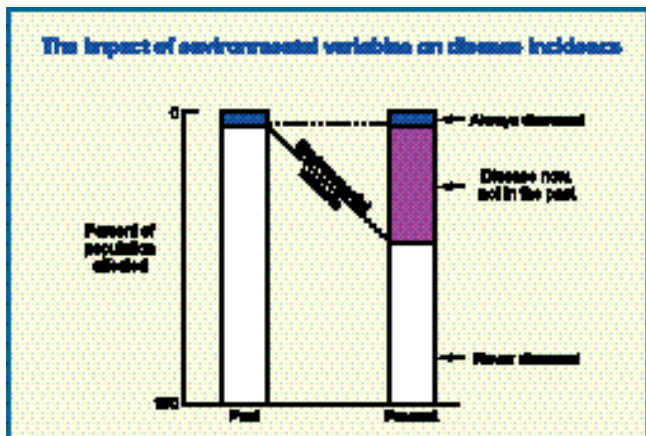


Figure 1. The impact of environmental variables on allergic disease incidence. There have always been patients with allergies in strongly genetically predisposed individuals. However there is now a larger subgroup of people who have allergic symptoms that they would not have had if they had been born a few decades ago. The cause must be an environmental change.

Table 1
<p>According to recent retrospective epidemiological studies, you are less likely to be allergic if:</p> <ul style="list-style-type: none"> • you have older siblings (boys better than girls) • you rarely washed your face and hands as a child • you have had infections via the faeco-oral route • you were brought up on a farm with animals • you keep a dog • the dust in your home is contaminated with bacteria • you lived in Communist rather than western Europe • you had early tuberculosis or BCG vaccine, and are tuberculin positive (but some conflicting studies discussed in Rook and Stanford, 1999) <p>You are more like to be allergic if:</p> <ul style="list-style-type: none"> • you were given antibiotics as a small child

evolved as hunter-gathers and agriculturalists, always close to earth and untreated water. Some organisms that are very common in mud and untreated water, such as mycobacteria, are rare on concrete or in the chlorinated water of the modern environment. Moreover, there is absolute proof that exposure to mycobacteria has been hugely reduced in rich Western countries.

Most individuals in developing countries show immunological reactivity to soluble antigens from environmental saprophytic mycobacteria injected into the skin. (This test is identical to the Mantoux test used to monitor exposure to *Mycobacterium tuberculosis*, a pathogenic member of the genus.) However, positive delayed hypersensitivity skin test responses to environmental mycobacteria are rare in developed countries. Similarly, studies of the bacterial colonisation of the guts of neonates have revealed that there is more rapid and diverse colonisation in developing countries, and that there is a continuing rapid turnover of species and strains that is not seen in the rich European countries.

Moreover, a direct comparison of racially similar Estonians and Scandinavians, with low and high incidences of allergies respectively, showed that the children in Estonia had significantly higher counts of *Lactobacilli* in their intestinal flora (Sepp *et al.*, 1997). From the point of view of the evolution of the human immune system, this is another interesting bacterial genus, because primitive man used many stored and fermented drinks and vegetables that were extremely rich in *Lactobacilli*.

Diseases on the rise

Before discussing the mechanism that renders exposure to environmental bacteria essential to our immune systems, we need to broaden the hygiene hypothesis to include two other groups of immunological disorder. It is not only allergies that are increasing. There is a parallel and closely correlated increase in the incidences of certain autoimmune diseases.

Type 1 diabetes, where the body destroys its own insulin-secreting b cells in the pancreas (Figure 2; Stene and Nafstad, 2001), and multiple sclerosis, where the immune system targets components of the nervous system, are both becoming more common. The inflammatory bowel diseases (IBD, Crohn's disease and ulcerative colitis) are also increasing at a comparable rate (Sawczenko *et al.*, 2001). They started as rare diseases, so incidences are still relatively low, but they are becoming more common in small children, where the long-term effects are dreadful. IBD's are due to inappropriate responses to the contents of the gut. The gut obviously contains food we have eaten, but the large bowel, in an adult, also contains approximately 10^{14} bacteria. It is clearly essential that our immune system does not generate inflammatory responses to these gut contents.

The overall picture that emerges is that, in the developed countries, we have rising incidences of at least three groups of disease that are due to inappropriate activation of the immune system. When the target antigens are the trivial levels of harmless allergens in the air (house dust mite, grass pollen, cat proteins, etc), we get allergies. When the target is a component of our own tissues, we get autoimmune diseases. When the target is in the gut contents, we get IBD.

The connecting link

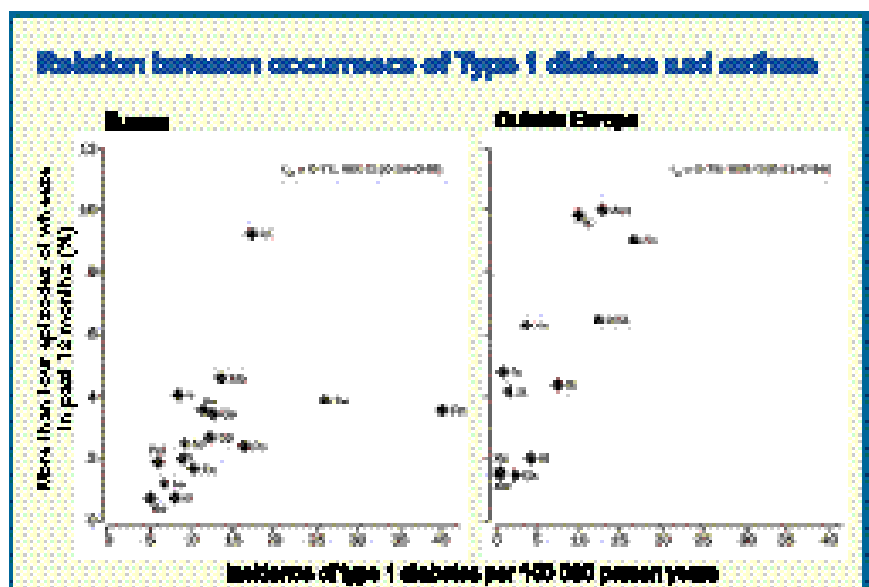


Figure 2. Parallel increases in the incidences of allergic disorders and Type 1 diabetes in rich countries, both within and outside Europe from Stene and Nafstad (2001).

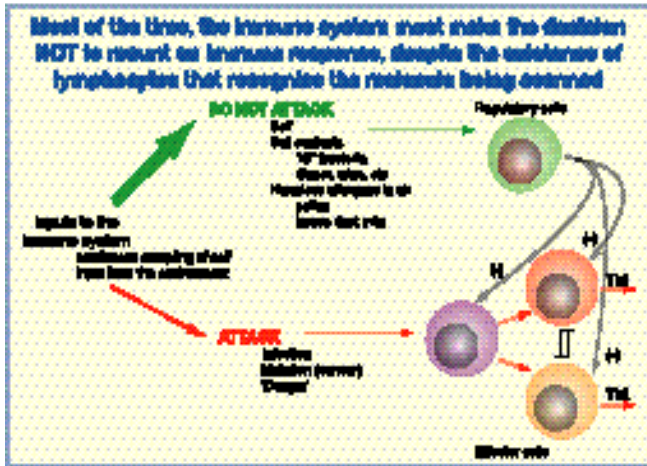


Figure 3. Effector and regulatory lymphocyte subpopulations. The immune system samples and screens the internal environment, and also material reaching the skin and mucosal surfaces. Most of the time an effector immune response (Th1 and/or Th2 cells) is NOT activated, and regulatory cells block the inflammatory response.

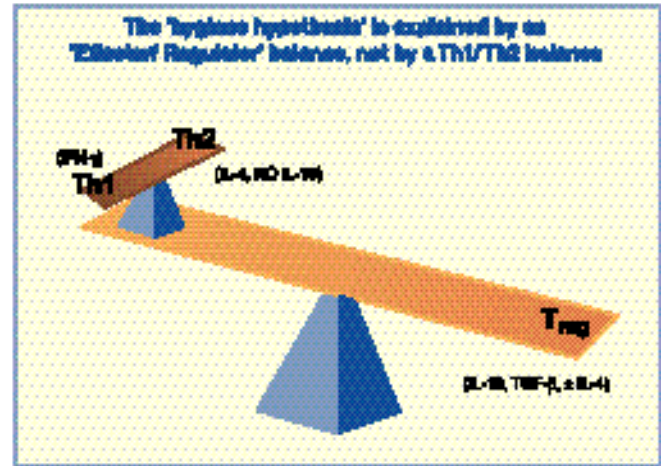


Figure 4. The crucial balance between regulatory and effector T cells. If regulatory cell activity is low, immunoregulatory disorders can occur. The nature of the disease manifested depends on genetic background and Th1/Th2 balance, determined by the individual's immunological history. (IL; interleukin. TGF- β ; Transforming Growth Factor Beta)

The connecting link between these disease groups is the fact that they all represent immune responses that should not occur. They are diseases of faulty regulation of the immune system. Contrary to the usual perception, the major task of the immune system is to know when NOT to respond. The system constantly samples and screens material from within our own tissues, and also from the exterior and, 99.99% of the time (an educated guess!), it must make the decision not to mount an immune response (Figure 3).

Most of this 'censoring' is achieved by a multitude of different types of regulatory cells (Read and Powrie, 2001). These work by preventing rather than promoting the activity of effector lymphocytes (Figure 3). They release inhibitory cytokines such as interleukin 10 (IL-10) and Transforming Growth Factor Beta (TGF- β). There is also direct cell-cell contact between regulatory T cells and the effector cells, or between regulatory cells and the antigen-presenting cells that process and present antigen to the receptors on effector T cells – though these interactions are not well understood. Mice whose IL-10 gene has been knocked out develop spontaneous severe IBD.

Therefore the current version of the hygiene hypothesis, formulated in 1998 (Rook and Stanford), is that the developed countries are suffering from an escalating epidemic of diseases of faulty immunoregulation (Figure 4).

Who gets which disease?

The major effector lymphocytes of the immune system can differentiate towards polarised forms that secrete distinct patterns of cytokines and other mediators. T helper 1 lymphocytes (Th1) secrete interleukin 2 (IL-2) and interferon gamma (IFN- γ). These cells are mainly involved in the autoimmune diseases that are increasing (Type 1 diabetes and multiple sclerosis). The real function of Th1 cells is the activation of macrophages, and protection from diseases such as cancer, tuberculosis and most viruses. On the other hand, Th2 lymphocytes secrete IL-4, IL-5 and IL-13. Th2 lymphocytes probably evolved mainly to provide immediate explosive reactions to helminths attempting to invade mucosal surfaces. It is when these immediate reactions target harmless aeroallergens that the common allergies arise. Different forms of IBD can be dominated by Th1 cells, or show a mixed pattern.

So who gets which disease of faulty immunoregulation? It is

known that, to some limited extent, Th1 lymphocyte activity down-regulates Th2 lymphocyte activity and *vice versa*. Thus, although Th1-mediated and Th2-mediated diseases involving different target antigens can co-exist, in several studies it has emerged that individuals with a Th1-mediated immunoregulatory disorder (Type 1 diabetes) tend not to also suffer from a Th2-mediated immunoregulatory disorder (allergies). Therefore, it appears that in individuals with faulty immunoregulation, the disease manifested will be determined by the genetic background of the individual and also by his immunological history. Th1/Th2 balance depends to a large extent on the types of infection or vaccination to which the individual has been exposed (Figure 4).

The prime primer

Germ-free animals born by caesarian section and maintained in a sterile environment have abnormal immune responses. For instance, it is not possible to induce immunological tolerance to allergens administered in high dose by the oral route in germ-free animals, though this crucial physiological pathway is restored if the gut is colonised with bacteria (Sudo *et al.*, 1997). Similarly, we have known for more than 30 years that the susceptibility to autoimmune arthritis is changed in germ-free animals and can be increased or decreased by colonising the gut with different bacterial species (Kohashi *et al.*, 1979). There appear to be several mechanisms involved.

T lymphocytes are generated in the thymus by random mutation of the antigen receptor molecules, followed by selection for ability to interact with thymic antigen-presenting cells that inevitably present self components (Figure 5). Very strongly anti-self T cells are eliminated and so are those that fail to interact with antigen-presenting cells at all. That leaves a repertoire of weakly anti-self lymphocytes. This method of generating a lymphocyte repertoire makes sense because of the evolutionarily determined antigenic interrelationships between all life forms. To put it simply: a lymphocyte that weakly recognises self stands an excellent chance of very efficiently recognising a bacterial version of a related protein, whereas random mutation of T cell receptors without such a selection step would produce vast numbers of T cells that recognise nothing found in this biosphere.

But clearly, T cells positively selected because they weakly recognise self are potentially dangerous to the host.

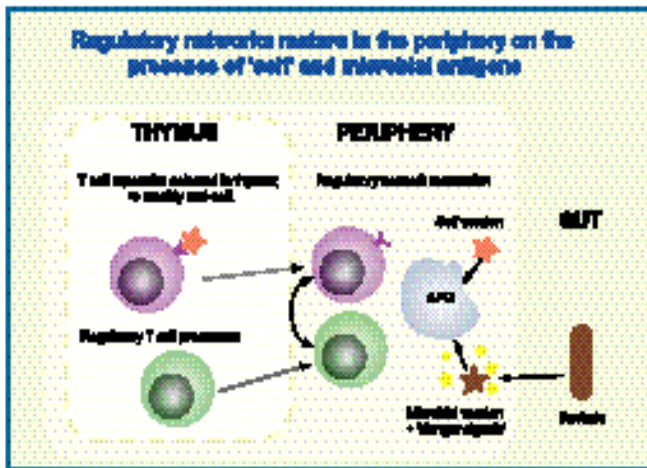


Figure 5. Random mutation of the antigen receptor genes of T cells in the thymus generates precursors of both effector T cells, which weakly recognise self proteins, and regulatory T cells. These cells must encounter the self proteins and the bacterial homologues in the periphery in order to develop a fully functional regulatory network that can discriminate between self and non-self.

Therefore, the thymus produces not only precursors of effector T lymphocytes, but also precursors of regulatory T cells. Then, after leaving the thymus and migrating to the peripheral tissues, effector T cells and regulatory T cells are exposed to self, and to bacterial versions of similar proteins (which are probably identified because of the simultaneous presence of other bacterial 'danger signals'). In complex interactions with antigen-presenting cells, the regulatory network is established (Figure 5). In other words, we all have T cells that potentially recognise our own tissues, but they are kept under tight regulation.

This is not the only mechanism for priming regulatory cells that requires bacterial products. There also seem to be bacterial components that have a pharmacological effect on antigen-presenting cells, so that they boost regulatory rather than effector responses. Moreover, regulatory cells are most efficiently primed against a somewhat Th1-biased background, perhaps because of a requirement for IFN-g. Again, bacteria are major inducers of Th1 responses.

Vaccines as educators of the immune system?

Since hygiene and antibiotics are effectively blocking our exposure to many environmental bacteria, childhood vaccinations might have taken over the 'educational' role. If they have, are they doing it right (Figure 6)? Vaccines have a huge impact because they are injected intramuscularly or subcutaneously. The rich city dweller has few injuries that will introduce bacteria into these sites, so the vaccine schedule may represent a significant fraction of the entire parenteral bacterial exposure. Moreover, vaccines are administered with powerful adjuvants, and they are heavily biased towards induction of Th2 responses, which is not good for induction of regulatory T cells. If the vaccination schedule is failing to activate enough regulatory T cells, the answer is not to abandon vaccines, but rather to develop vaccines that **do** prime regulatory cell networks.

Exploit microorganisms

If the increase in diseases of faulty immunoregulation is due to decreased exposure to certain microorganisms, it ought to be possible to use the bacterial species concerned as protec-

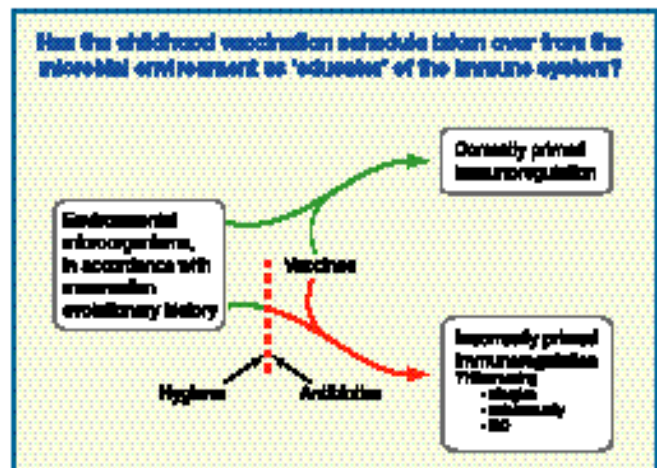


Figure 6. Hygiene and antibiotics are depriving the immune system of an essential microbial input. In the rich countries, the childhood vaccination schedule might replace this input. But does it do the job correctly and prime the right regulatory cells?

tive or therapeutic vaccines against these diseases in animals and man. Similarly, it should be possible to show that the mode of action is via the induction of regulatory T cells. The genus *mycobacterium* is the most studied, but *Lactobacilli* and helminths have received some attention too (Yazdanbakhsh, 2002).

Live or killed mycobacterial vaccines will prevent subsequent induction of allergies in experimental animals and, more importantly, they can be used to treat a pre-existing allergic state. Even the Bacillus Calmette Guérin (BCG), which is used as a vaccine against tuberculosis in many countries (though not in the USA), may have some ability to exert these effects though there are conflicting data. Mycobacterial preparations have also been able to alleviate autoimmune diseases in experimental animals.

These mycobacterial preparations turn out to work by induction of regulatory T cells. When allergic animals are treated with SRP299 or SRL172, vaccine preparations derived from an intensively studied saprophytic environmental mycobacterium, the allergic state is attenuated, and a population of regulatory T cells develops in the spleen. These T cells can be transferred intravenously to other allergic animals, thereby suppressing allergy in the recipients too (Zuany-Amorim *et al.*, 2002). Simultaneous administration of neutralising antibodies to IL-10 and TGF- β shows that these cytokines are involved in the suppressive effect (Figure 7). Experiments of this type confirm the overall hypothesis and open the door to clinical trials in human subjects.

Other approaches include the use of live organisms that may colonise the gut (probiotics), or the 'farming' of the gut flora by consuming materials that favour the growth of the desired species. Recently, a clinical study of the administration of *Lactobacilli* to neonates at risk for allergies has also yielded encouraging results (Kalliomaki *et al.*, 2001). Several other studies have looked at SRP299 or SRL172 in patients with seasonal or perennial asthma, or with atopic eczema (Arkwright and David, 2001). Overall, the results are encouraging and larger studies are now in progress.

Conclusions

Diseases attributable to inappropriate activity of the immune system are increasing rapidly. The increase can be traced to environmental changes that are resulting in failure to prime the control mechanisms within the immune system (Yazdanbakhsh, 2002). This is exciting because solutions to the

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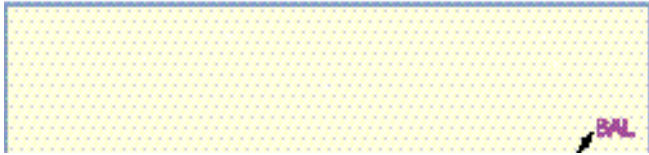


Figure 7. Treatment of allergic mice with a mycobacterial vaccine, SRP299, leads to the development of allergen-specific regulatory T cells that can suppress allergic manifestations in an allergen-specific manner that involves IL-10 and TGF- β , when transferred intravenously into allergic recipients.

problem involving changes to vaccination schedules are rapidly emerging.

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