



Forward Look

Gene-Environment Interaction in Chronic Disease



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Dr Nathalie Spielwoy, ESF, France (until Dec. 2010)

Science writer:

Dr Iain Patten, Spain

Coordinator:

Ms Nicole Stirnberg, ESF, France

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Foreword



Over the last half century a dramatic increase in the incidence of chronic inflammatory diseases such as asthma, allergy and irritable bowel syndrome has rightfully led to concern about how the modern lifestyle may inappropriately trigger innate physiological defence mechanisms. Solid evidence from decades of epidemiological and laboratory studies supports the notion that a significant gene-environment interaction underlies this phenomenon. However, despite a clear linkage between lifestyle and genetic background, a thorough understanding of the underlying mechanisms and how these complex and chronic diseases are triggered and progress is only just beginning to emerge.

The societal challenges associated with inflammation and immune-mediated pathologies are evident. As pointed out by the World Health Organization (WHO) as well as by other health monitoring organisations, the socio-economic costs of chronic diseases are staggering and ever increasing. There is therefore an urgent need to prioritise resources and identify the most efficient scientific and societal initiatives to be adopted within this area. In this context, national collaboration within the European region to establish the state of the art and to promote an efficient exchange of best practices is essential. For chronic diseases, such an approach likely represents the most efficient manner in which strategies for amelioration of the increase of chronic diseases in the western world may be achieved.

The present report, *Gene-Environment Interaction in Chronic Disease (GENESIS)*, results from an ESF Forward Look initiative led by the European Medical Research Councils (EMRC) under the auspices of the European Science Foundation (ESF). Experts from industry and

academia as well as from relevant interested organisations have been consulted in the process of conducting this initiative and have, based on this work, developed a set of final recommendations.

The report describes the state of the art from epidemiological and scientific perspectives and identifies the way forward through a set of clear scientific and policy-relevant recommendations. Adoption of a synergetic research approach that involves industry and academic research as well as policy level regulations at the national and European levels will provide the most efficient manner in which to address and tackle the grand challenges that the increased emergence of chronic disease represents in Europe.

Professor Liselotte Højgaard,
EMRC Chair

Professor Marja Makarow,
ESF Chief Executive

Executive Summary



Changed lifestyle conditions such as improved nutrition and exercise status have contributed to a significantly increased longevity in Western Europe. However, evidence indicates that changes in living conditions also play an important role with respect to the alarming increased incidence rates that have been observed for chronic diseases.

The complex interplay between genes and environment in chronic inflammatory and immune-mediated diseases is generally not well understood. Based on genetic predisposition, environmental factors largely determine phenotype development. In this context, the complex gut-associated microbial ecosystem (gut-microbiota) for example and nutrition-related factors are known to be important environmental triggers for the development and modification of lifestyle-related chronic diseases and metabolic pathologies, and the hygiene hypothesis provides a further challenging concept for the explanation of increases in chronic inflammatory diseases. The inflammation and immunological dysregulation that likely underlies the emergence and disease progression of most chronic diseases is clearly linked to complex gene-environment interactions, the detailed nature of which still needs to be characterised at the cellular and molecular levels.

Developments in sequencing technologies over the last decade include the possibility to perform genome-wide association studies (GWAS). These technologies have allowed the linkage of small genetic susceptibility factors and analyse their contribution to the development of, or protection from, chronic disease. Furthermore, insights within the area of gene activation studies, also known as epigenetic regulation, have added to our understanding of how external environmental stimuli may trigger cellular responses. Genetic/epigenetic research

at the cellular level in combination with a better understanding of cellular response may therefore significantly contribute to improving our understanding of the underlying disease pathophysiology.

The mucosa linings of the gut and the airways represent important natural barriers between intrinsic regulation and the environment. Induction of adverse signalling in and responses of these cells in response to external pathogens is therefore central to the development of chronic disease. At the cellular level, responses of the endoplasmic reticulum (ER) and other sub-cellular organelles may be central to the induction and progression of various chronic inflammatory diseases, including inflammatory bowel disease (IBD), Crohn's disease, atopic eczema, allergy and asthma. The involvement of epithelial barriers in mucosal immunity in this context may represent a target where an interaction between tissue inflammation and the development of adverse immunity to external pathogens may be connected. Further, a better understanding of such cellular events may generate an improved understanding of how early events during the development of the immune system may be involved in establishing chronic disease. The manner in which such systems are linked at cellular and systems levels is presently not clear and therefore represents future research areas where analyses of cellular response with immune mediated reactions may be integrated.

The microbiome, often referred to as the forgotten organ, reciprocally interacts with the host and thereby plays a vital role in both health and disease. Research into the influence of this 'organ' on the development and progression of chronic disease is urgently needed to better understand the complex relationship between extrinsic and intrinsic pathogenic pathways in chronic disease. The appropriate

temporal and spatial resolution of such studies will be centrally important in order to improve our understanding of how interactions between the microbiome and the host may lead to chronic inflammatory diseases; in particular the importance of this interaction during early childhood is relevant in this context. In many ways, the appropriate understanding of chronic inflammatory disease and immune-mediated disease may start with a better understanding of how we interact with the surrounding microbiome.

Preventive measures to chronic disease initiation and disease progression have been studied for several years. Inflammation and immune-suppression are among the strategies that to some extent have shown some therapeutic effect. However, the issues of prevention have proven to be more difficult to address, partly due to the complexity and lack of understanding of the disease process. Probiotic compounds that may interact and modulate the microbiota represent an example of a combined therapeutic and preventive strategy. The efficacy of such preventive approaches is controversial, which may reflect the complexity of the microbiome. Other preventive measures include the use of dietary medications, which for several chronic disease areas including diabetes is well supported by epidemiologic research. However, how these may be understood in the context of cellular responses is an area of major research interest.

The present report outlines the state of the art of the ongoing research within the interconnected areas of extrinsic, intrinsic and therapeutic preventive research in chronic disease. Based on this in-depth presentation of the scientific issues, the report addresses the grand challenges associated with the disease areas. These areas can generally be associated with therapy and prevention, large scale cohort studies, partnerships, research tools, data generation and handling and infrastructure and personnel. Based on a thorough evaluation of each of these areas the present report outlines how each area may be involved and contribute to the future of research into chronic disease. A set of 10 recommendations was developed. These recommendations both target researchers at the individual level and indicate how research into chronic inflammatory disease may be prioritised by policy measures in the coming decades.

Consensus recommendations

The delegates in Berlin identified the following ten key areas as having the highest priority for research into chronic inflammatory disease:

1. Research should distinguish clearly between therapy and prevention
2. Large prospective cohort studies including deep phenotyping should be made a priority
3. Strategies and tools should be developed to ensure adequate sampling of the environment
4. A global (international) approach should be taken to understanding chronic inflammatory disease
5. Effective interdisciplinary research strategies must be established
6. New tools and experimental models must be developed
7. Protocols for data collection, handling, and storage need to be harmonised
8. Substantial investment must be made in infrastructure, personnel, and development of research tools
9. Dedicated funding must be provided for interdisciplinary research
10. Effective public-private partnerships must be developed to ensure free exchange of information

1.

Introduction



In the last half century, Europe has witnessed a dramatic change in the profile of human health. Enormous advances have been made in the prevention and treatment of disease, to the extent that many transmissible diseases have now been effectively eradicated. In parallel, however, there has been a marked rise in the incidence of chronic disease, particularly inflammatory diseases such as asthma, allergy, and inflammatory bowel and other autoimmune diseases.

In the context of an ageing population, the overall healthcare burden may be rising once again, to such an extent that we might now question whether the impact of health improvements achieved in the 20th century is beginning to be reversed in the 21st century. In order to respond effectively to this worrying trend, strategies are urgently needed to determine the causes of chronic disease and identify targets for therapy and prevention. To facilitate agreement on future priorities, the European Science Foundation therefore initiated a Forward Look entitled Gene-Environment Interaction in Chronic Disease. This exercise brought together experts from a range of relevant fields to define the grand challenges facing research into chronic inflammatory disease and agree on recommendations for the future of research in the field. Inevitably, given the complexity of this effort, not all relevant chronic inflammatory diseases have been included in depth and selections have been made to illustrate the recommendations.

Since the 1950s, the incidence of chronic inflammatory diseases has increased almost exponentially in industrialised countries. Conditions such as asthma, Crohn's disease, type-1 diabetes, and multiple sclerosis have increased by as much as 400% in many regions. The link between the occurrence

of these diseases and the changes in lifestyle and living conditions in the West has led many of them to be considered diseases of affluence. This notion is supported by the observation that countries such as China, India, and members of the former Eastern Bloc – which are adopting an increasingly Western lifestyle in line with growing affluence – are now showing signs of a similar trend in the incidence of these diseases.

The factors responsible for the development of chronic inflammatory disease are not easy to tie down. While epidemiological evidence clearly points to an environmental influence, not all individuals in these environments develop disease. Furthermore, the range of different pathological findings that can fall under the umbrella of a single diagnosis is quite striking, suggesting that different individuals, with apparently similar susceptibility and exposed to the same environments, may nevertheless display substantial clinical variation. Individual susceptibility must thus play a role in determining not only disease propensity but also disease course and pathological characteristics. Understanding the interaction between these intrinsic and extrinsic mechanisms of chronic inflammatory disease will be the key to their future treatment and prevention.

Susceptibility to chronic inflammatory disease has a clear genetic component. However, genetics may not be the only determining factor. Prior to birth, maternal nutrition and exposure to pollutants and tobacco smoke in utero, for instance, can produce epigenetic changes that act alongside genetic predisposition to determine the biological response to environmental stimuli after birth. These prenatal exposures can also influence later susceptibility to disease via effects on the morphogenesis of tissues and organs. For instance, the branching of the

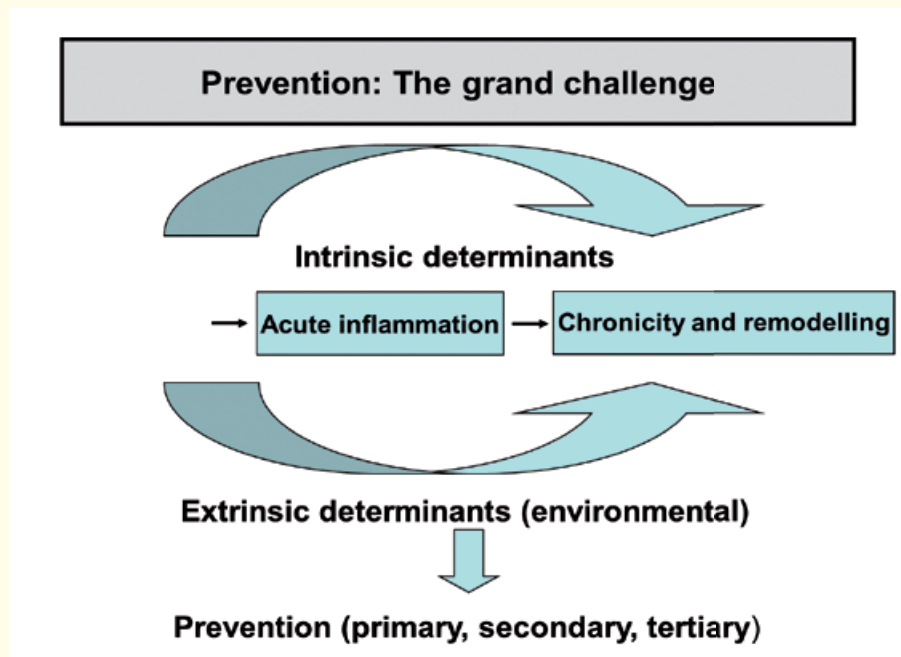


Figure 1.

Development of chronic inflammatory disease is a dynamic event. Following initiation, the disease process involves acute inflammation that moves towards chronicity and tissue remodelling as well as regeneration. These events are controlled by both intrinsic and extrinsic determinants. Grand challenges in this area are the development of prevention (primary, secondary and tertiary) as well as development of causal therapies.

airways to form the lungs in the developing fetus can modulate the later susceptibility of the child to develop asthma. After birth, other factors such as breastfeeding and exposure to microorganisms appear to further influence the likelihood of developing diseases such as asthma and allergy. Thus, from the point of conception, and perhaps even before, intrinsic and extrinsic factors interact to determine the probability that an individual will be healthy or develop a chronic disease given the right environmental stimuli.

The environmental stimuli to which the human organism is exposed following birth are varied and constantly changing. One component of the environment that is thought to play a central role in chronic inflammatory disease is the community of microorganisms that we carry around with us on our skin and in our gut and airways. Interestingly, the composition of these microbial populations appears to influence whether or not susceptible individuals are protected against chronic inflammatory disease. Children who grow up in environments containing complex microbial populations, for instance, may have some degree of protection against diseases such as atopy and asthma. In fact, the diversity of microbial exposure during childhood has been shown to be inversely related to the likelihood of asthma. These and other factors such as nutrition thus make attractive targets for future prevention strategies. But before disease prevention can become a realistic goal, much more needs to be known about which factors protect against or initiate disease, how they act, and when. In the gut, for instance, many hundreds of different organisms are present in a delicate ecological balance. Yet we still do not

know which of those organisms have a specific influence on immune or metabolic processes in health or disease, or indeed the effects of altering individual organisms on the ecology of the gut.

Once chronic inflammatory disease has been initiated in a susceptible individual, it follows a complex course that gradually becomes irreversible. Inflammation is a protective response of the immune system to harmful stimuli, yet in these diseases it gradually becomes maladaptive. The factors that determine this shift remain poorly understood but the outcome is irreversible inflammation and tissue changes that ultimately lead to a poorly functioning organ. Yet most chronic inflammatory diseases do not follow an invariant course. Instead, periods of relative clinical stability are punctuated by periods of exacerbation in which symptoms worsen before stabilising again, usually without returning to the previous baseline. Importantly, the environmental factors that trigger these exacerbations may also influence susceptibility to subsequent insults. Thus, as in the case of prenatal influences on organ and tissue susceptibility, environmental exposures at a given point in an individual's life may profoundly influence pathology many years down the line.

Given this level of complexity, is it realistic to consider a future in which chronic inflammatory diseases become treatable and even preventable? There may be reason for hope. Alongside the rapid changes in the epidemiological profile of chronic inflammatory diseases, substantial progress has also been made in biomedical research. Perhaps the most significant advance that has occurred in the last 20 years is precisely our ability to measure and analyse

complexity. The incredible developments in so-called omics technologies, for instance, have made it possible to describe an individual's biological composition to a level of detail that was previously unimaginable. We are now in a position to obtain something akin to a biological fingerprint containing comprehensive descriptions of a person's genetic makeup, metabolism, and physiology.

Alongside the generation of these vast datasets on the intrinsic factors that influence the pathophysiology of these diseases, similar efforts are underway to describe the extrinsic components. For instance, complete phylogenetic descriptions have now been generated for the microorganisms populating the gut (the microbiome) and researchers are now beginning to determine the extent to which different microbial profiles are linked to specific disease phenotypes. Nevertheless, many hurdles need to be overcome if omics approaches are to fulfil their potential. Most notably, advances are still needed in our ability to interpret the enormously complex data sets that are generated. Methods are also required to validate conclusions from the perspective of systems biology. Omics technology merely identifies associations that do not necessarily reflect a cause and effect relationship. Consequently, this hypothesis-generating research must inevitably be followed by focussed, hypothesis-testing studies to establish causality.

European healthcare research is now faced with a significant challenge if it is to meet the needs of its population in the decades ahead. The tools with which we hope to generate meaningful insights into the intrinsic and extrinsic mechanisms of chronic inflammatory diseases must be adequately exploited and further developed in order to identify treatment and prevention strategies. This will require significant and integrated investment in research, without which the effectiveness of European healthcare will be weakened. To maximise the potential of future healthcare strategies, insights will be required from a number of different fields, including epidemiology, genetics, cell biology, immunology, and bioinformatics, all feeding ultimately into effective clinical research. The following three sections describe a selection of topics in which major scientific progress has been made and grand challenges identified for the future during three strategic workshops held in Barcelona in October 2010. These insights fed forward into a Consensus conference held in Berlin in March 2011 to agree on general recommendations for future European research into the mechanisms of chronic disease and strategies for their treatment and prevention.

2.

Intrinsic Mechanisms Underlying Chronic Inflammatory Disease



The increasing incidence of chronic inflammatory disease observed in industrialised countries is clearly linked to environmental and lifestyle factors. Nevertheless, not all individuals respond similarly and there is evidence of familial inheritance in a number of diseases. Consequently, diseases such as asthma, allergy, and inflammatory bowel disease (IBD) are widely considered to be a due to combination of environmental and individual risk factors. In addition to identifying environmental triggers, then, research into chronic inflammatory disease must determine the mechanisms underlying the response to those factors and the events that lead to disease chronicity.

Genetics

The starting point for any exploration of intrinsic mechanisms in chronic inflammatory disease is invariably genetics. Although epidemiological evidence clearly supports a role for environmental triggers in diseases such as allergy, asthma, and IBD, it is apparent that the pathogenesis is also dependent upon genetic susceptibility. The advent of genome-wide association studies (GWAS) thus raised the hope that disease risk could be stratified according to individual susceptibility alleles. Unfortunately, the situation has proved to be substantially more complex, and simple genetic explanations for individual susceptibility to chronic inflammatory disease have not been forthcoming.

GWAS to identify loci conferring genetic susceptibility to IBD have yielded more than 60 genes linked to Crohn's disease and 40 to ulcerative colitis. Despite many of these genes having known roles in key mechanisms such as innate immunity,

autophagy, and inflammation, most of the risk alleles are very frequent in the healthy population. In some cases, it appears to be the major alleles that confer susceptibility.

Similar observations have been made in diabetes. Despite clear evidence of heritability in type-1 diabetes (T1D), the common polymorphisms identified in GWAS only explain less than 30% of the heritable risk. The situation in type-2 diabetes (T2D) is even more striking. Here, having a first-degree relative with T2D is associated with a hazard ratio of between 3 and 4 for development of the disease compared with individuals who do not have affected relatives. Despite this clear heritability, the polymorphisms identified by GWAS only account for around 1% of T2D heredity, making them essentially useless for risk prediction.

GWAS in allergic diseases are even more difficult to interpret because the definition of disease varies from one study to another. It has, however, become clear that there are separate genetic polymorphisms which affect susceptibility to allergic sensitisation and totally different genes are associated with the susceptibility to allergic diseases such as asthma or eczema. The disease-specific genes are mostly associated with epithelial function, while those for allergy affect the balance of immune responses.

These findings highlight a key challenge in defining the genetic factors that determine disease susceptibility in chronic inflammatory disease, namely the failure of single alleles or even allele combinations so far to facilitate clear risk prediction. It is important to note, however, that the lack of clear associations with common single-nucleotide polymorphisms (SNPs) does not mean that these genetic differences do not underlie susceptibility. Individual SNPs with small effects may together have meta-

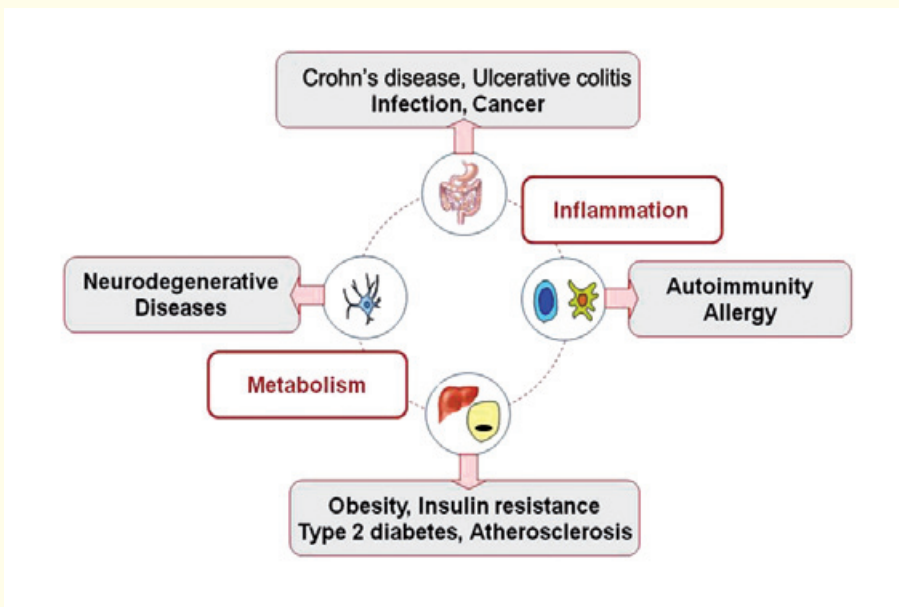


Figure 2. Metabolic and immune-mediated pathologies with inflammatory processes in various target organs as the key driver of disease initiation and/or perpetuation. Extrinsic and intrinsic events (red) lead to the manifestation of disease (black).

bolic consequences that can be used as biomarkers in risk stratification. Sequencing technology has advanced to such an extent that it can now be considered trivial to analyse 1500 SNPs at a time. The real challenge for future research into genetic susceptibility to chronic inflammatory disease may lie in the ability to discern patterns from the data obtained, and this will depend upon our ability to interpret highly complex datasets. Furthermore, genetic variation is not only about SNPs. Other factors such as repetitive DNA elements or copy-number variants could also be involved.

The concept of genome-wide association requires that the comparators be clearly defined. In other words, we must be clear about the nature of the specific disease phenotypes with which associations are being made. Much of the focus of the studies undertaken to date has been on associations with late stages of disease. In order to fully understand the role of genetic susceptibility in the development of chronic inflammatory disease, however, much more information will be required on the phenotypes of all stages, from initiation, through propagation, to the onset of chronicity. Increasing the temporal resolution of disease phenotypes should also be accompanied by improved classification of the diseases themselves. Many inflammatory diseases are less clearly defined than we would like to imagine and it is increasingly apparent that they often form a continuum. By increasing the resolution of phenotyping in chronic inflammatory diseases, we will be better placed to define clearer associations between intrinsic susceptibility, environmental inputs, and the relationship between the two in the transition from initiation to chronicity.

Epigenetics

Epigenetic modifications, namely those heritable characteristics of the genome other than DNA sequence, may play a crucial role in chronic inflammatory disease by providing a link between early environmental influences and the development of chronic disease later in life. Comparison of methylation patterns between individuals exposed to severe prenatal famine and their unexposed siblings, for instance, has shown that transient prenatal exposure to a specific environmental stimulus leads to persistent epigenetic changes. This raises the hope of elucidating the links between exposure to environmental factors and subsequent susceptibility to either the development of disease or the transition from an acute to a chronic disease phenotype.

Epigenetic signatures might serve as biomarkers for specific prenatal environments, and genome-wide studies have the potential to reveal biologically significant changes. In order to achieve these goals, however, future studies will need to obtain a reliable catalogue of prenatally induced epigenetic signatures associated with adverse events or environmental exposures. It will also be important to obtain robust evidence that these signatures have a causal relationship with the pathogenesis of the disease. Finally, epigenome-wide association studies will need to develop a framework that accounts for biological complexity.

A crucial element of epigenetic studies will be the generation of tissue- and cell type-specific data. One example in which evidence has been gained for the epigenetic regulation of specific cell types is in the differentiation of T-helper (TH) cells from

naïve CD₄⁺ lymphocytes. These immune cell types are linked to autoimmunity and allergy, and they may be central to the development of chronicity by generating a long-lasting cellular memory of earlier environmental exposures. Methylation of the IFNG (interferon gamma) promoter has been identified as a marker for TH₁ lineage commitment. Thus, in a specific immune cell type, epigenetic changes can determine important cell fate decisions that are likely to influence disease. One of the major challenges will now be to identify the signals that influence such cell type-specific changes, assess the role of these changes in the pathogenic mechanisms underlying defined phenotypes, and, ultimately, to determine whether they can be manipulated therapeutically.

As in genomic analyses, obtaining an overview of epigenetic associations will require more detailed information on disease phenotype, including temporal changes from initiation through to chronicity. In addition, however, consideration of epigenetics raises another key challenge. Epigenetic differences must be associated not only with disease phenotype but also with the environmental inputs that generate them. Determining how to sample the impact of the environment, however, is perhaps the single greatest challenge for research into environment-gene interactions.

Ultimately, of course, it will also be necessary to achieve a mechanistic understanding of the effects of epigenetic modifications in normal physiology and disease. Epigenetic changes can either increase or decrease gene expression. For instance, direct hypermethylation of DNA will silence genes while modifications such as methylation of histones can increase expression. Such changes in gene expression may underlie the association between the use of folic acid during late pregnancy and an increased risk of asthma in offspring. Folic acid is a potent methyl donor and epigenetic changes arising from its use may cause silencing of TH₁-associated genes such as IFNG. Thus, the effect of folic acid-induced epigenetic modifications during pregnancy may underlie cellular changes that increase individual propensity to asthma. Another epigenetic mechanism that has begun to receive much attention involves the regulation of post-transcriptional gene expression in the cytoplasm by micro-RNAs. The transcription of micro-RNAs is affected by environmental factors, and they are also present in human milk in an acid resistant form, which suggests a potential role in modulating immune maturation in infants.

Cellular Mechanisms

A key focus for research into the cellular mechanisms underlying chronic inflammatory disease is the tissue-environment interface. It is here, particularly in the mucosa lining the gut and airways, that a careful balance must be maintained between host defense and uncontrolled inflammation.

The identification of genetic or epigenetic associations with disease phenotypes facilitates risk stratification and can therefore help to guide monitoring and prevention. In order to convert these observations into an understanding of the disease process and, ultimately, to develop therapeutic strategies, however, experimental approaches must be used to determine the cellular mechanisms involved.

Substantial advances have now been made in our understanding of the role of microbial sensing, autophagy, and endoplasmic reticulum (ER) stress in the pathogenesis of IBD. The innate immune system contains a large array of receptor molecules that recognise conserved microbial structures and initiate cell signalling cascades. This signalling maintains both physiological host-microbial homeostasis and epithelial barrier integrity. However, it may also lead to the induction of an inflammatory reaction accompanied by tissue disruption and organ dysfunction. A better understanding of the signalling events occurring in the intestinal epithelium in both healthy and disease states will provide critical insights into the processes that maintain gut homeostasis and prevent an inappropriate inflammatory reaction to the colonising commensal microbiota while simultaneously allowing the induction of an inflammatory host response in the event of enteropathogen infection. Key signalling pathways downstream of innate immune receptors include the interferon, mitogen-activated protein kinase, and NF- κ B pathways. The transcription factor NF- κ B is linked to an enormous range of disease processes. Tissue-specific activation of the transcription factor in the gut epithelium of mice leads to spontaneous colitis. Interestingly, however, this genetic effect is dependent upon the gut microbiota, since mice reared under germ-free conditions remain healthy. Thus, expression of the inflammatory disease phenotype is clearly determined by a gene-environment interaction, most likely involving a response of the epithelium to the gut microbiota.

A number of genetic risk factors for IBD converge on the Paneth cell. Paneth cells produce antimicrobial peptides, endogenous antibacterial effector molecules that critically contribute to maintaining the epithelial integrity. Interestingly, one such risk allele of the *Atg16l1* gene, which is involved

in autophagy and is linked to Crohn's disease, requires an environmental insult in order for the disease phenotype to be expressed. When susceptible cells are infected with norovirus, they become activated and develop a hyperreactive status, but this is not converted into chronic inflammation. Thus, at least two gene-environment interactions appear to be necessary to initiate the disease and then progress to a chronic inflammatory phenotype. However, the nature of the environmental factor that triggers chronicity remains unclear. In addition, the circuits regulating the function of Paneth cells and the biology of antimicrobial peptide-mediated host defence are poorly understood.

The unfolded protein response is a pathway from the ER to the nucleus that protects cells from stress caused by unfolded or misfolded proteins. Interference with this pathway in mice, for instance by knocking out the transcription factor *Xbp1*, results in an IBD-like phenotype. Closer analysis reveals that the gut epithelium lacks Paneth cells and, to a lesser extent, goblet cells. These are the most secretory cell types, and therefore most susceptible to ER stress. When ER stress cannot be controlled in these cells, however, they become unable to respond appropriately to their environment or to fulfil their role in managing the microbial milieu. Many of the genes involved in the regulation of ER stress are also linked to autophagy and to bacterial sensing. For instance, Nod proteins control autophagy by recruiting *ATG16L1* to the plasma membrane at the site of bacterial entry and influence bacterial handling and antigen presentation in dendritic cells.

Atopic eczema is another example of a disease associated with an epithelial barrier defect. Polymorphisms in the *filaggrin* gene, which encodes a protein that binds skin epithelial cells, are a relatively common risk factor for eczema. The defective barrier is susceptible to water loss and infection, and forms a route of sensitisation to environmental allergens. Allergy increases the severity of the eczema and the risk of progressing to other allergic problems such as asthma, hay fever, and even food-induced anaphylaxis. Despite *filaggrin* not being expressed in the airway epithelium, polymorphisms are also associated with an increased risk of asthma. This may represent a genuine allergic march in which a defect allowing sensitisation to aeroallergens through the skin leads ultimately to allergic asthma.

Elucidating the cellular mechanisms underlying the loss of function at epithelial interfaces is a key challenge for the understanding of the intrinsic mechanisms that influence chronic inflammatory

disease. In normal homeostasis, these pathways allow epithelial barriers to fulfil their role in sensing and responding to the surrounding milieu. Genetic risk factors can predispose the cells to become hyperreactive in response to environmental insults, but additional factors are required to convert this response into chronic inflammation. Future research should focus on defining the mechanisms through which genetic risk factors lead to loss of homeostasis and generate mucosal inflammation, and on identifying and characterising the protective and pathogenic environmental factors that encourage homeostasis and promote inflammation, respectively. More detailed phenotyping may help us to define individual diseases and their pathogenic time course in more detail but, in order to design experimental studies that test causality, more information about the physiological situation is also required. It is also important to understand experimental models in the context of human physiology rather than in isolation. Defining the differences between mice and men will be of critical importance in ensuring that insights from the laboratory can be transferred to the clinic. Perhaps one of the grand challenges for the next five years, then, is to lay the foundations for truly integrated research projects in which clinical and epidemiological, hypothesis-generating research provides the basis for experimental, hypothesis-testing research in appropriate models that allow findings to be transferred as smoothly as possible back to clinical settings.

The key cell types that influence the development of chronic inflammatory disease fall into two broad categories, epithelial cells and cells of the immune system. Much interest has been generated by the role of the epithelial cells in the barrier function of the epithelium lining the gut, airways and skin. The mechanism by which these cells influence the development of chronic inflammation, however, is relatively poorly understood and few clear data have been obtained *in vivo*. An important challenge will be to define the complex interactions between the epithelial barrier and mucosal immunity and to address the impact of the microbiota on epithelial cell functions. The critical importance of this question is illustrated by the dramatic change in the gut epithelium from a sterile and largely restricted site *in utero* to an environmentally exposed body surface that is densely colonised by bacteria after birth. The processes that facilitate this transition and influence both the bacterial composition of the microbiota and the maturation of the epithelium and underlying immune system to promote stable, life-long homeostasis are largely undefined. These include signalling pathways that promote epithelial

homeostasis and integrity and prevent an inappropriate inflammatory response under physiological conditions but also help to mount an efficient anti-bacterial host response in the event of infection.

In the gut, the lamina propria located beneath the epithelium is the largest reservoir of immune cells in the body. Mucosal dendritic cells and macrophages play a key role in host defence, yet despite their proximity to luminal bacteria, mucosal inflammation is controlled under normal physiological conditions. This is thought to be largely regulated by the suppressive cytokines transforming growth factor β (TGF- β) and interleukin (IL)-10, which downregulate the proinflammatory activity of the macrophages in a process referred to as inflammation anergy.

Besides functions classically attributed to dendritic cells and macrophages, there is accumulating evidence to suggest that intestinal macrophages modulate T cell responses to luminal antigens. Oral tolerance describes the acquisition of immune hyporesponsiveness to ingested innocuous substances and depends primarily on a finely tuned cross-talk between innate immunity (antigen-presenting cells) and adaptive immunity (T cells), as well as on the integrity of the epithelial barrier in the gut. Experiments in mice have led to the proposal of a multi-step model of oral tolerance in which dendritic cells from the gut mucosa migrate to the mesenteric lymph nodes (MLNs) to induce a seed population of gut-homing T cells. Gut homing allows T cells to enter the lamina propria and to interact with local intestinal macrophages which constitutively secrete IL-10 and drive further expansion of tolerance favouring regulatory T (Treg) cells. This suggests that intestinal dendritic cells and macrophages might turn out to be key players in regulating tolerance and immunity induced via the intestine.

A body of evidence suggests that there are certain windows of opportunity for tolerance induction, especially the neonatal period and probably also late in fetal life. Reprogramming of the immune system may in fact be a lifelong process. Naturally occurring Treg cells with immunosuppressive properties are abundant in human fetal MLNs, and in mice it has been shown that neonatal (but not adult) CD4+ T cells are prone to differentiate into Treg cells upon stimulation. Homing of Treg cells to the human gut mucosa seems to be particularly active in infancy.

As alluded to above, our mechanistic understanding of the cellular activities underlying the induction of oral tolerance is based primarily on mouse experiments. It is believed that CD103+ migratory dendritic cells, and perhaps also mac-

rophages, carry antigen from the gut to MLNs, where they promote Treg -cell induction, particularly under the influence of TGF- β and retinoic acid derived from vitamin A. Additionally, subepithelial non-migratory CD103- CX3CR1+ macrophage-like cells produce IL-10 that contributes to expansion of the Treg cells when they have homed from MLNs to the lamina propria of the gut. The homing of Treg cells may also target peripheral tissues and organs, and this has raised hope of preventing or curing inflammatory disorders through dietary supplementation with appropriate antigens. Although this approach has been successful in experimental animals, human trials have been disappointing except for some promising results, mainly in the field of food allergy.

The increasing prevalence of allergic disorders in infancy suggests that the underlying immune dysregulation is most likely to be an early event. Experiments based on cord blood cells suggest that this may be ascribed to abnormal functioning of dendritic cells and Treg cells in an immunologically immature environment, with an impact of genetic factors or epigenetic changes induced in the intra-uterine environment. In addition, there are many biological variables linked to the establishment of an adequate postnatal epithelial barrier, including the generation of secretory immunity. Secretory IgA inhibits inappropriate immunological activation by so-called immune exclusion. In this process, secretory IgA at the epithelial surface restricts colonisation of microorganisms and impedes penetration of agents that could cause hypersensitivity reactions or infection. Finally, the role of the large, phenotypically diverse population of intraepithelial intestinal T cells in immunoregulation and defence remains uncertain.

Understanding the development of tolerance will be crucially important in the design of treatment and prevention strategies for chronic inflammatory disease. There is good evidence that introduction of substantial amounts of gluten prior to 4 months of age significantly increases the risk of coeliac disease, and similar observations have been made in relation to introduction of weaning foods and the development of food allergy. However, some observations have suggested that delaying the introduction of gluten until after 6 months also increases coeliac disease risk. Furthermore, there is indirect evidence from several studies that delayed introduction of highly allergenic foods beyond 6-9 months of age is likewise associated with increased risk of food allergy. As the World Health Organisation recommends that weaning only commence after 6 months, there is an urgent need to conduct studies to establish whether

in an affluent environment such as in Europe, this advice is appropriate or not. One hypothesis is that overlapping weaning with continued breast feeding has the best chance of achieving tolerance. Studies are therefore needed to improve our understanding of the factors in human breast milk that modulate the immune response in infants. While human milk contains a remarkable array of immune modulators including secretory IgA, they vary in quantity over time in the same mother and also considerably amongst women. Identifying the environmental factors that affect the quality of human milk could thus yield targets for intervention to improve protective effects and influence the development of tolerance.

Organs and Systems

Chronic inflammatory disease is influenced by a variety of cell types, not only those found in the epithelia and mucosal immune system. This is highlighted most clearly in the interaction between the nervous system, the immune system, and the microbiota. Mice reared under germ-free conditions show evidence of an exaggerated stress response compared with those reared under specific pathogen-free (SPF) conditions. Notably, this effect can be attenuated by provision of individual bacterial species or faeces from SPF mice. However, this can only be achieved in a short window of opportunity during early development. Likewise, many of the long-term effects of maternal separation in rats can be prevented by ingestion of specific commensal bacteria. Thus, the establishment of the microbiota in neonatal life directly influences neural function.

Just as diet and gut commensal bacteria can influence neural function, so the brain can influence immune mechanisms and the host microbiota. Stimulus of the vagus nerve can interrupt the effects of lipopolysaccharide via effects on macrophage production of tumour necrosis factor. Likewise, vagotomised animals have an exaggerated response in acute DSS colitis. Stress can also have major effects on the microbiota, and this will itself have consequences for inflammatory disease.

These factors must be taken into account when interpreting data from both longitudinal and interventional studies. An additional future challenge, then, is to ensure that factors such as maternal and infant stress are controlled in interventional studies and, where possible, monitored in cohort studies as a factor that may influence the sensitive balance between host-related and environmental factors that will determine future disease susceptibility.

Looking Forward – the Grand Challenges

Next-generation GWAS

The initial findings of GWAS have failed to identify alleles that fully explain disease heredity. However, the focus to date has been on individual alleles and common SNPs. The next phase of genetic studies will need to look at combinatorial effects, as well as other factors such as repetitive elements and SNPs occurring in regulatory sequences. Sequencing technology has advanced to such a degree that data generation can now be considered trivial. The real challenge will be to undertake meaningful analyses of those data in order to identify new associations that may provide clues for research into disease mechanisms.

Refining the models

The likelihood that genetic susceptibility to chronic inflammatory disease is dependent upon complex gene interactions, variation in non-coding sequences, and epigenetic signatures raises serious challenges for the development of models in which to establish causality and elucidate mechanisms. Current animal models based on single gene changes may be inadequate to address the complexity of these multigene effects. Similarly, animal housing conditions optimised to reduce experimental variability (i.e. defined commensal microbiota, absence of pathogens and defined diet) present problems for research aimed at understanding the environmentally modulated course of disease. Effective organotypic models of human epithelial surfaces must be developed and used to understand the cell biology of the host. Consensus is urgently needed on how to obtain suitable models in which to analyse the mechanisms of chronic inflammatory disease.

Redefining the phenotype

The genetic similarities observed between different diseases raise the possibility that our phenotypic definitions of disease are inadequate or possibly even inaccurate. It is conceivable that by reversing the logic of GWAS we may find previously unexpected phenotypes. Thus, by taking genetic associations and looking for biological correlates, we may achieve a radical redefinition of accepted phenotypic classifications. This challenging possibility could open up an entirely new understanding of the continuum of inflammatory disease.

Mapping the pathways

A great deal still remains to be understood about the signalling events that determine the balance

between physiology and pathology in chronic inflammatory disease. Careful mapping of the signalling pathways employed by the innate immune system, for instance, in specific disease phenotypes, under healthy physiological conditions, and in response to specific stimuli will generate important insights into the intrinsic mechanisms underlying individual disease states. This is an area in which continued support for independent academic research activities undertaken by small, competitive research groups is likely to be of major importance. Such approaches are not only productive but also cost-effective and likely to generate new insights that will drive the translational research of the future.

Key points

To achieve a mechanistic understanding of chronic inflammatory disease, major efforts are still required to:

- Characterise the interplay between disease susceptibility genes in clearly stratified disease phenotypes
- Identify cell- and tissue-specific epigenetic signatures in populations at increased risk of disease
- Define the impact of environmental exposures on epigenetic marks across the life course, including an assessment of the role of inflammation and metabolism on prenatal programming of disease
- Describe the cell- and tissue-specific changes that characterise the progression towards chronic disease
- Define the role of bacterial signals at mucosal surfaces such as the gut and lung that interfere with tolerance induction or immune-mediated disease progression

References

1. Xavier, R.J. & Rioux, J.D. Genome-wide association studies: a new window into immune-mediated diseases. *Nat. Rev Immunol* **8**, 631-643 (2008)
2. Nathan, C. & Ding, A. Nonresolving inflammation. *Cell* **140**, 871-882 (2010)
3. Portela, A. & Esteller, M. Epigenetic modifications and human disease. *Nat. Biotechnol.* **28**, 1057-1068 (2010)
4. Garrett, W.S., Gordon, J.I., & Glimcher, L.H. Homeostasis and inflammation in the intestine. *Cell* **140**, 859-870 (2010).

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Extrinsic Mechanisms Underlying Chronic Inflammatory Disease



A simple look at the geographical distribution and temporal trends in the incidence of chronic inflammatory diseases reveals clear evidence of an environmental influence. Diseases such as IBD and asthma have risen sharply in the last 40 years in parallel with industrialisation and urbanisation. The spread of these diseases seems to follow lifestyle factors such as nutrition, hygiene, and exposure to environmental microbial components.

At the body surfaces, which form the interface between the organism and its environment, lies the microbiome. This forgotten organ has a complex reciprocal relationship with its host. Its composition and activity can have both beneficial and deleterious effects, and at the same time, the physiological state of the organism can modulate the profile and impact of the microbiome. Both host and microbiota are of course also influenced by environmental factors. Thus, the microbiome may be a true intermediary in the interaction between the organism and its environment, and it has become a key focus of research into the extrinsic mechanisms that determine the transition from health to chronic disease.

The microbial cells in the gut outnumber the human cells in the body by 10 to 1. Furthermore, there are 150 times as many genes in the intestinal metagenome as in the human genome. Perhaps even more striking is the degree of genetic variation among the microbes of the gut. Whereas the genome of any two human beings differs by only 0.1%, that of any two *E. coli* from the gut is likely to differ by around 40%, and these bacteria represent only a tiny proportion of the gut microbiota. Thus, the potential for variation among the microbial symbionts living in the transition zone between body and environment is orders of magnitude greater than that of the organisms on which they live.

Characterising the Microbiome

A detailed description of the microbiome will require data on the genomic profiles, phylogenetic relationships, functional properties, and spatial and temporal distribution of the different microorganisms that it comprises. Each of these elements presents a variety of technical and logistic challenges.

The approaches used to characterise the microbiome can be broadly divided into two categories: those that are dependent upon the ability to culture the microorganisms and those that do not require cultivable isolates. Genomic and other omic (transcriptomic, proteomic, and metabolomic) approaches are not culture dependent, and vast amounts of information can be obtained directly from samples of the microbiota. Work by the MetaHIT (Metagenomics of the Human Intestinal Tract) Consortium, for instance, has led to the generation of a catalogue of 3.3 million bacterial genes, accounting for 85% of abundant gut-microbiota genes from a cohort of 124 Europeans and 70% to 86% of genes from groups in the United States and Japan. This reference gene set will be used in combination with genomic data to search for associations between microbial genes and disease, and preliminary findings suggest that individual species of gut microbes can fully discriminate obesity and IBD phenotypes. This initial extensive research effort aimed at characterising the gut microbiome will pave the way for future studies into other important sites of bacterial colonisation such as the skin, vagina, and respiratory tract.

The observation that certain bacterial species in the gut microbiota are associated with specific IBD phenotypes raises the question of whether

these organisms are causal agents, contributors, or a consequence of the disease. The functional analyses required to discriminate between these possibilities, however, are largely dependent upon the availability of cultivable isolates. Yet the vast majority of microbes cannot currently be cultured. A key challenge, then, in translating the data obtained from metagenome-wide association studies into experimentally testable hypotheses will be to extend the range of cultivable isolates beyond the small number currently available.

Increasing the availability of cultivable bacterial strains will also provide tools with which to address factors such as substrate preferences and competition. These factors are likely to be crucial in determining the composition of the microbiota at different locations on the host organism – such as the skin, respiratory tract, and gut, and even at specific sites within each – and at the same site in different individuals. Thus, insights into the ecology of individual species will be essential if we are to understand the significance and consequences of differences in the microbiome.

Spatial resolution

Phylogenetic analyses based on microbial sequence data have begun to yield important insights into the spatial organisation of the human microbiome. By tagging the microbial DNA sequences present in different samples with a sequence barcode, it is possible to assess the phylogenetic similarity of the microbes present at different locations on the body, between different groups of subjects, and in different disease states. Interestingly, host-associated communities such as those of the human gut, skin, vagina, etc. show a very strong phylogenetic clustering, much stronger than that of samples from different terrestrial or marine environments. Thus, each individual area of the body appears to represent a discrete ecological niche that determines which organisms are selected from an apparently more phylogenetically homogeneous environment. This raises important questions about how the different niches are defined and maintained, how the microbiota develops in each region, and what consequences variation in this microbial ecology may have for human disease.

A first step toward answering these questions will be to generate detailed spatial descriptions of the human microbiome. For instance, the challenge of obtaining samples from different levels of the human intestine has meant that characterisation of the gut microbiome has so far been based largely on bacteria present in faeces. More detailed information is also going to be needed in other areas of the

body surface, however, most notably in the airways.

Despite widespread suggestions that the respiratory tract forms a sterile environment, sampling of the airways, including the lungs, reveals that the numbers of bacteria are similar to those found in the upper intestine. The profile of the most prevalent species differs according to the anatomical level of the airway samples (e.g. oropharynx and upper lobes of the lungs). Comparison of the microbiota in the same region of the airway between healthy individuals and patients with respiratory diseases such as asthma or chronic obstructive pulmonary disease also reveals clear differences, both in adults and children. For instance, compared with healthy individuals, patients with respiratory disease have larger numbers of known respiratory pathogens. In contrast, anaerobic species appear to be much more common in healthy airways. Interestingly, the difficulties associated with culturing anaerobes may at least partly explain the belief that the airways are essentially sterile.

Obtaining a detailed spatial description of the human microbiome presents a number of important challenges for future research. Sequencing is now more or less trivial but the information obtained is critically dependent on the methods used to collect and handle samples. A number of groups, including the MetaHIT Consortium, have begun to develop standard operating procedures for sample handling in studies of the microbiome, but it is likely that much more work will need to be done in order to ensure consistency and, therefore, interpretability of results. In addition to these technical challenges, the influence of host genetic differences and environmental exposures must also be taken into account. A question for future studies, then, is which information needs to be collected and how this should be done in order to maximise the data that can be obtained and minimise duplication of effort. Furthermore, as we begin to describe the microbial populations at spatially restricted sites in more detail, it will be important to integrate genomic, transcriptomic, and metabolomic data on the microbial communities with specific host responses, epithelial biology, and immune function. Given the highly dynamic nature of these interactions, quantitative analysis will need to be applied.

Development and maintenance of the microbiota

A key determinant in the development of the human microbiota is likely to be exposure at birth. Analysis of the microbiota of newborn infants within 20 minutes of delivery reveals that, while its initial

composition is essentially uniform across different body sites, there are substantial differences between infants according to delivery route. Whereas the microbiota of vaginally delivered infants is similar to that of the mother's vagina, the microbiota of infants delivered by cesarean section is similar to that of the mother's skin. Although this observation may not be entirely surprising, when we consider the high level of specificity in the composition of the microbiota from different adult body regions, its implications are significant. Any factors that alter the maternal microbiota on the skin or vagina around the time of birth could have a profound impact on neonatal colonisation. Furthermore, these changes cannot be assumed to be uniform. Maternal antibiotic use, for instance, may have a different effect on vaginal microbial populations than on those of the skin, whereas the skin microbiota may be more susceptible to change based on environmental contact. Achieving a more detailed description of human microbial ecology will thus be a prerequisite to understanding the factors that influence the early infant microbiota and its effect on subsequent disease. Section delivery has been associated with a higher risk of allergic disease and particularly food allergy in some individual studies, but the balance of evidence suggests no correlation between mode of delivery and incidence of allergic diseases.

The impact of exposure to environmental factors on the intrinsic mechanisms of chronic inflammatory disease may thus act in part through the microbiome. For instance, in the non-obese diabetic (NOD) mouse model of T1D, the cumulative incidence of diabetes is higher in female than in male animals when reared under SPF conditions. When NOD mice are reared in fully germ-free conditions, however, this sex bias disappears. Furthermore, early exposure to a single commensal species in NOD mice reared under germ-free conditions confers protection against T1D. Thus, differences in exposure to commensal bacteria interact with intrinsic factors such as sex to determine the expression of a pathogenic phenotype. The influence of the microbiota on the development of disease, however, is not unidirectional. Evidence suggests that the microbiome of NOD mice differs from that found in diabetes resistant (NOR) mice. Exactly how these genotype differences influence the microbial environment remains to be determined. Furthermore, the significance of this difference for the development of T1D will require further study.

A key challenge in determining how environmental exposure influences the development of the microbiota is to achieve reliable sampling. Exposure

to individual species or groups varies substantially over time and there is greater species heterogeneity during particular periods of the year. As a result, the between-measure sampling variability can be quite high and there is a substantial probability of underestimating or failing to identify associations between exposure and disease. Future studies should therefore focus on adequate repeated sampling, banking of environmental and biological samples, and careful standardisation of methods.

Despite these technical and logistic challenges, significant advances have already been made in our understanding of how early environmental exposure to microbes influences the risk of subsequent disease. One of the clearest examples is seen in the effect of farming environments on the development of allergy and asthma. It is apparent from epidemiological studies that the shift towards an urban lifestyle is linked to increasing prevalence of immune-mediated disorders. Nevertheless, in many European regions, comparison of disease prevalence in urban and rural environments yields inconclusive results. When the prevalence is assessed in rural environments alone, however, there is a very clear difference between children from animal-farming and non-farming families. Thus, the animal-farming environment represents a natural model in which to evaluate the effect of environmental exposures on allergic outcomes.

Children born in animal-farming families have a substantially reduced risk of allergy and asthma, and the effect appears to be due to the diversity of the environmental microbes to which they are exposed and seemingly also to components (polysaccharides) of the fodder. Interestingly, analysis of markers for innate immunity, which are upregulated in school-age children from farming families, suggests that the strongest influence comes from maternal exposure to farm animals during pregnancy. The more types of farm animal the mother is exposed to, the greater the effect on innate immunity in the children.

This natural human model provides a great opportunity to identify the specific environmental factors that are responsible for the protective effect of prenatal and infant exposure to farming environments. Also, since some children born in farming families do still develop allergy and asthma, it may be possible to investigate the genetic and epigenetic mechanisms underlying the response to protective environmental exposures. The challenge highlighted by this example will be to design cohort studies in which information can be obtained on both environmental and host-related factors alongside detailed clinical monitoring and even mechanistic

studies using the samples obtained. In this way, it might be possible to identify specific associations that provide insights into the relationship between the intrinsic and extrinsic mechanisms underlying chronic inflammatory disease.

The role of the microbiota is not restricted to early childhood. Although initial colonisation of niches like the gut and, possibly, the airways is likely to have a determining effect on the profile of the adult microbiome, other systemic and environmental influences can also be expected to play a role in the dynamics of the microbial populations. One example is in the complex relationship between diet and the gut microbiota. As would be expected, diet has a clear influence on the microbial populations found in the gut. However, the microbiota also influences factors like energy uptake and the effects of metabolic byproducts such as butyrate. This may explain, for instance, the observation that low carbohydrate, high protein diets are associated with an increased risk of colorectal cancer. Under these conditions, there is a substantial reduction in butyrate levels coupled with an increase in genotoxic metabolites. Although these changes are linked to reductions in the numbers of butyrate-producing bacteria in the gut, there is substantial interindividual variation. The role of vitamin D has also attracted substantial attention in relation to the influence of nutritional intake in allergy and autoimmune disease. Treg cells express vitamin D receptors, and some epidemiological studies have linked vitamin D deficiency with increased risks of asthma. Similar relationships have been found for reduced intake of other dietary components, such as fresh fruit and vegetables and omega-3 polyunsaturated fatty acids, factors that may well interact with genetic and epigenetic variation. An important challenge for future research, then, will be to begin to unravel the complex population dynamics of the microbiota in response to environmental inputs such as nutrition and to explore the role of host niche-related factors in determining bacterial responses.

Mechanisms of Host-Microbiome Interactions: Establishing Causality

There is perhaps no clearer illustration of the relationship between extrinsic and intrinsic mechanisms than that seen in the interaction between host tissues and the commensal microbiota. Evidence has accumulated to support reciprocal interactions between the host immune system and bacteria living on the body surfaces. Consequently, one of the

major challenges for our understanding of the influence of host-microbiome interactions on chronic disease is to determine when changes in the microbiota are a primary cause of disease and when they reflect the adaptive capacity of bacteria in response to changing ecological conditions, for instance the influence of mucosal immunity.

An important consideration for any research into the role of the microbiome is to determine whether changes precede the onset of disease or are merely a consequence of it. Data on this point, mostly based on faecal microbial cultures, are currently limited and often conflicting. Nevertheless, some evidence has been obtained to suggest that the diversity of the microbiome in infants, particularly in relation to the balance of bifidobacterial species, is associated with increased risk of subsequent atopic dermatitis and allergic sensitisation. Although much less is known for asthma, there is evidence of differences in the metagenome of the lower respiratory tract between asthmatic patients and healthy controls. Also, colonisation with *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, *Haemophilus* has been reported to precede new-onset asthma in a birth cohort study.

A key issue for the understanding of how the microbiome can influence chronic disease is its effect on systemic immune responses. Support for a systemic influence of the microbiota comes not only from epidemiological studies but also from experiments in animal models. It is known, for instance, that the normal gut microbiota has a protective effect against the development of T1D in the NOD mouse model. In different disease models, however, the effect of the microbiota or its components may be deleterious or there may be no effect at all.

Contradictory findings have also been reported regarding the ability of the microbiota to influence systemic immune responses in immunocompetent animals. For instance, some authors have argued that the peripheral immune effects of the microbiota are restricted to the relevant compartment (the intestine), while others have shown that bacterial factors can translocate from the gut to the bloodstream to activate immune cells. In addition, the results of some studies have suggested that peripheral immune responses are only stimulated in animals with defects in innate immunity, suggesting that the host immune status itself influences the response to the microbiota.

Despite the complexity of the problem, some advances have begun to be made in identifying the cellular and molecular mechanisms through which the microbiota can influence the mucosal immune system. Bacterial flagellins, for instance, have

been found to have species-specific effects on gene expression in epithelial cells via binding to toll-like receptors (TLRs). Notably, induction of NF- κ B, a key event in the development of inflammation, is found to be flagellin dependent. Thus, epithelial cells appear to be responding to bacteria directly by activating signalling pathways with known roles in inflammatory disease.

When the response to flagellate and aflagellate pathogens is analysed in mice, clear differences in dendritic cell recruitment are observed. Rather than triggering normal recruitment, aflagellate salmonella lead to severe intestinal inflammation and the development of granulomas in the small intestine. This suggests that bacterial signalling through the epithelial cells is involved in regulating the mucosal immune response. Impaired epithelial signalling through the flagellin/TLR pathway results in an abnormal immune response and severe, chronic inflammation.

Such findings raise important questions about the role of the mucosal barrier under normal physiological conditions. It is widely assumed that the mucus layer serves to prevent bacteria from accessing the epithelium except under conditions of disease or damage. However, further research is clearly needed to determine whether this is truly the case or whether bacteria are in fact able to access the epithelium of a healthy gut (or airway). Notably, for bacteria to have an impact on the host it may only be necessary for soluble components to penetrate the mucus (and epithelial) layer and interact with TLRs.

Attempting to address the interaction between the mucosa and the microbiome is a major technical challenge, not least because the gut microbiome detected in faecal specimens may not represent the organisms which have the greatest impact on epithelial events. Mucosal biopsies may ultimately be required to truly identify the pivotal interactions between organisms and the human host. Furthermore, since the microbiota varies according to the environment in which animals are raised, reproducibility of results obtained at different research centres is an important consideration. One possible solution is to consider the use of multisite trials in the same mouse strains. Great care must also be taken in the choice of controls, since unintentional variations in the microbiota must be limited as far as possible. Establishing causality is central in any attempts to understand the role of extrinsic factors in the mechanisms of chronic inflammatory disease. Descriptive studies of the microbiome will be essential for generating hypotheses about the protective or pathogenic role of microbial species, but these hypotheses must

eventually be tested in defined experimental models. Gnotobiology, or the rearing of animals under germ-free conditions, can then be used to analyse the role of the microbiome or its component species either under normal physiological conditions or in specific animal models of human disease.

The combination of precisely defined genetics and controlled microbial exposure represents a powerful tool with which to explore the relationship between intrinsic and extrinsic mechanisms. In principle, it should be possible to introduce individual bacterial species or species combinations into animals with defined and even tissue-specific genotypes in order to analyse the consequences for global, tissue-level, or cellular disease phenotypes. As with all experimental models, however, the results obtained will be dependent upon the parameters. Key challenges will be to ensure that the models accurately reflect the human phenotype and that the dynamics of the microbiota mimic those of the corresponding human microbial niches. Ongoing efforts will be required to increase our understanding of the complexities of the human phenotype, both under normal physiological conditions and in disease, and to use these findings to generate the most appropriate experimental models.

Looking Forward — the Grand Challenges

Functional analyses

Genomic and phylogenetic analyses of the microbiome will provide important insights into the relationship between disease states and variations in the species profile of the microbiota. If studies are designed appropriately, it should also be possible to analyse the associations between these factors and the genetic and epigenetic variations apparent in the host. This will require the use of well-defined gnotobiotic animal models. However, in order to improve our understanding of the microbiome and perhaps begin to manipulate it therapeutically, we will also need to carry out functional analyses of the components of the microbiota. An important challenge for microbiome research, then, will be to increase the availability of cultivable isolates and assess the factors that influence their ecology in healthy and diseased hosts. New paradigms exceeding the Koch-Henle postulates need to be defined and developed to create a new scientific basis for functional studies. This information will also be essential in interpreting the results of manipulating individual species within a complex microbiota.

Environmental sampling

Assessment of environmental impact is limited by the extent to which we are able to measure the environment. The sequencing of the human gut microbiome has raised hopes of microbiome-wide association studies, but it will now be important to extend the principle to other organs (e.g. the skin and airways) and environmental exposures. Even in something as apparently straightforward as nutrition, experience has shown that accurate, reliable information on individual food consumption is hard to obtain. The challenge, then, must be to develop paradigms for accurate sampling of the environment. Efforts must be made to determine which environmental factors to sample, when, and how.

Integrated longitudinal studies

The influence of early exposure to farming environments on the risk of allergy and asthma is an example of where long-term cohort studies are expected to yield substantial insights into the extrinsic mechanisms of chronic disease. By combining additional genetic and epigenetic data on the subjects, along with careful collection of environmental and biological data, such studies could provide further clues as to the relationship between intrinsic and extrinsic mechanisms. Is it time, however, to consider extending these approaches to include an embedded mechanistic component? As samples and data are generated, close collaboration with laboratory research groups could allow hypotheses to be tested at an earlier stage while retaining the open-minded approach characteristic of epidemiological studies. Substantial logistic and economic barriers will need to be overcome through common strategic goals if such a long-term integrated approach is to be successful. In addition, however, improvements in statistical methods will be required to reduce the complexity of data on the diversity of the microbiome to manageable but biologically meaningful dimensions.

Key points

To better understand the impact of environmental exposures and the extrinsic mechanisms underlying chronic inflammatory diseases, significant research effort will be required to:

- Accurately sample the relevant environmental exposures, in particular the environmental microbiome
- Generate a detailed spatial and temporal profile of the microbial populations found in the environment and at specific sites on the host
- Achieve functional characterisation of the host-microbe interaction
- Examine the effect on tolerance of modifying colonisation and exposure
- Establish long-term, multidisciplinary cohort studies that include careful phenotyping of enrolled subjects and comprehensive collection of environmental and biological data along with an embedded mechanistic component

References

1. Sartor, R.B. Microbial influences in inflammatory bowel diseases. *Gastroenterology* **134**, 577–594 (2008).
2. Slack, E. *et al.* Innate and adaptive immunity cooperate flexibly to maintain host-microbiota mutualism. *Science* **325**, 617–620 (2009).
3. Qin, J. *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).
4. Costello, E.K. *et al.* Bacterial community variation in human body habitats across space and time. *Science* **326**, 1694–1697 (2009).
5. von Mutius, E. & Vercelli, D. Farm living: effects on childhood asthma and allergy. *Nat. Rev. Immunol.* **10**, 861–868 (2010).

4.

Emerging Concepts in Therapy and Prevention



As we increase our understanding of the intrinsic and extrinsic mechanisms underlying chronic inflammatory diseases, we are faced with a new challenge, namely to use that knowledge to benefit human health. Insights into the response of the organism to environmental insults and the factors that determine the transition to disease chronicity will help us to define new therapeutic targets. Likewise, identification of the environmental factors that promote or protect against future disease raise realistic hope of prevention. The challenge for researchers is now to develop strategic approaches that will convert chronic inflammatory diseases into preventable and treatable conditions. Any such approaches will need to take into account the fact that the association studies that inform them do not necessarily imply cause and effect and that animal studies may not extrapolate to humans. Consequently, it is only through interventions targeting putative mechanisms that causal relationships can finally be demonstrated in humans.

Therapy

While it is to be hoped that insights into the intrinsic and extrinsic mechanisms underlying chronic inflammatory disease will lead to the identification of increasingly specific therapeutic targets, there is inevitably a lag between research and clinical development. In Crohn's disease, for instance, many of the newest drugs approaching the market target TH1-mediated immunity, which was thought up until recently to be central to the pathogenesis of the disease. The elucidation of the role of NOD2, however, has shifted attention away from a pri-

mary problem in adaptive immunity and towards defects in the innate handling of bacterial components. Clearly this highlights the ongoing need to streamline the translation of research findings into clinical development, but it also raises other issues. For instance, does our current knowledge of disease phenotypes and individual susceptibility support the search for a single effective pharmacological target? Experiences from the development of drugs to treat asthma suggest not.

IL-13 was identified as a key therapeutic target in asthma based on its ability to influence TH2 cell differentiation, airway inflammation, and hyper-responsiveness. Although therapeutic dosing of anti-IL-13 in mice clearly inhibited allergen-induced TH2 airway inflammation and remodelling, the results in human clinical trials were disappointing. In fact, the clinical control of asthma did not differ between placebo and anti-IL-13 treatment at any of the doses tested. Does this mean, however, that IL-13 is not an effective target for the treatment of asthma after all? Investigation of subsets of asthma patients would seem to suggest not.

Analysis of the expression of IL-13-inducible genes in the airway epithelium reveals that there are two major subphenotypes of asthma: TH2-high and TH2-low. Patients with TH2-high asthma have upregulated expression of IL-13 targets, increased bronchial hyperresponsiveness, and a positive response to corticosteroid treatment. While these steroid-responsive asthmatic patients also display other markers of classical asthma, such as eosinophilia and elevated IgE, patients with TH2-low asthma do not. On this basis it is an important observation that there is a statistically significant effect of anti-IL-13 therapy when assessed in a stratified population of asthmatic patients.

Grouping patients together without paying adequate attention to possible subphenotypes may lead to a failure to recognise or approve effective treatments. One such example may be sodium cromoglycate, which was removed from the list of drugs approved by the World Health Organization on the basis of a meta-analysis that showed no evidence of treatment benefit in children aged between 3 and 18. Given that approximately 50% of patients in fact have TH₂-low asthma, it is possible that sodium cromoglycate is indeed effective when used in the right patients. Thus, failure to pay attention to subphenotypes may have resulted in the loss of a safe and effective treatment option for asthma.

The need to ensure that potential treatments are assessed in the appropriate patient group is further exemplified by the results of clinical trials to assess the efficacy of anti-IL-5 therapy for asthma. IL-5 was another strong candidate target due to its role in eosinophil maturation, and experiments in animal models clearly supported IL-5 inhibition as a potential asthma treatment. Despite reducing eosinophilia, however, anti-IL-5 monoclonal antibodies did not produce a statistically significant improvement in asthma symptoms in clinical trials. Thus, as with anti-IL-13, the initially promising results of preclinical studies did not translate into clinical efficacy when tested in patients with asthma.

When asthmatic patients are selected on the basis of failure to control eosinophilia with high-dose oral corticosteroids, however, anti-IL-5 therapy reduces circulating and sputum eosinophils and leads to a 50% reduction in exacerbations. Although this subtype of steroid-refractory disease is observed in only 0.02% of patients with asthma, the drug is clearly effective when appropriately targeted. Thus, choice of patient group based on accurate phenotyping is a key concern for future drug development.

Experiences with anti-IL-13, anti-IL-5, and perhaps other drugs like sodium cromoglycate for the treatment of asthma highlight two key issues. Firstly, much more information is required on the range of phenotypes currently encompassed by a given disease classification. Without this sort of deep phenotyping, it will be impossible to determine whether a potential therapy is clinically ineffective or simply inappropriately targeted. Secondly, the experimental models used to identify putative drug targets must be sufficiently well characterised to determine whether they adequately reflect the human disease. Thus, both patient selection criteria and choice of animal model will be dependent upon improving our classification of specific disease phenotypes. If these advances can be made,

personalised therapy may prove to be a natural consequence of effective clinical development.

Pharmacogenetics is another example of the genotype-phenotype interactions driving a move towards personalised medicine. Certain genetic polymorphisms are associated with poor responses to either corticosteroids, leukotriene receptor antagonists, or agonists of beta-2 adrenergic receptors. These data clearly indicate that not all patients with the same disease phenotype benefit equally well from certain therapies. Furthermore, with increasing availability of biological drugs, it is very likely that the issue of therapeutic responsiveness will become increasingly important. These examples further highlight the need to develop diagnostic tests in parallel to the development of stratified treatment strategies that take into account individual therapeutic responsiveness. In order to identify useful clinical predictors, however, very strong associations need to be identified. Thus, odds ratios of 10 or more for the association between the predictive factor and the outcome are necessary (compared with associations about 1 log smaller that are usually found in GWAS) and this will require very large sample sizes to achieve adequate statistical power.

Prevention

Probiotics

The link between prenatal or perinatal exposure to complex microbiota and protection against allergic diseases raises the hope that provision of specific bacterial strains as infant milk supplements could be used in the prevention of these diseases. To date, however, the results have been mixed. While some studies have shown a reduction in the risk of diseases such as atopic dermatitis, others have failed to reproduce these findings. As a result, systematic reviews have concluded that the evidence is insufficient to recommend the use of probiotics as infant food supplements. Cochrane reviews of probiotics for the prevention of eczema and allergy, for instance, indicate only a weak, if any significant, effect.

The failure to obtain consistent support for the efficacy of probiotics does not necessarily imply that they no longer have a future, however. As with clinical trials of new asthma therapies, the challenge may be in defining the parameters. All probiotic strains are unique, and the results obtained with a given preparation will inevitably depend upon the specific strain used. Consequently, when meta-analyses consider probiotics as a whole rather than as individual strains produced under controlled conditions, the

results may be misleading. Furthermore, the efficacy and safety of a probiotic will also be determined by a range of host-related and environmental factors that must be carefully controlled if reliable conclusions are to be drawn from clinical studies. Nevertheless, the gut microbiome is extremely diverse and we have only a very limited understanding of the biology of many of the different strains present. Consequently, administering large doses of only one or two selected probiotic strains may prove to be too simplistic an approach. Before reliable prevention strategies can be developed, it is likely that much more research will be needed to understand the function of gut microbes in normal physiology and disease.

European Union regulations now require that the health effects and safety of a probiotic strain must be demonstrated in each case and that the strains must be clearly identified and deposited in public culture collections to facilitate comparison and verification. It is hoped that these regulations will have an immediate effect on public health by prohibiting the sale of a large number of bacterial preparations that are currently marketed without any clear demonstration of health benefits. However, it may also offer the longer-term benefit of promoting standardisation of strain characterisation and documentation of individual health promoting properties and safety. Future efforts should thus focus on quality control for the strain properties used originally as selection criteria for probiotics, as industrial processes may cause changes in the properties of live organisms.

The first major challenge for the future of research into probiotics will be to identify the changes in the microbiota that are associated with disease. In order to facilitate effective translation into clinical research, however, it will be essential to obtain extensive data on the context in which those changes occur. Thus, epidemiological studies must make every effort to rigorously record crucial information not only on environmental exposures but also on genetics and host-related factors. Furthermore, it will be necessary to explore the relationships between different strains of bacteria and between complex mixtures and the environment. It is only with this information that the results of clinical testing can be fully interpreted and appropriate, tailored interventions designed.

The potential use of probiotics is not limited to modulation of the microbiota. Both prevention and treatment of a variety of chronic diseases might be amenable to the use of genetically modified bacteria designed to deliver recombinant therapeutics. Experiments using *Lactococcus lactis* have begun to show promising results in both animal models and human subjects.

Evidence supporting the prophylactic use of recombinant probiotics has come from studies in patients with oral mucositis. This extremely debilitating condition involves painful inflammation, necrosis, and ulceration of the oral mucosa as a consequence of radio- or chemotherapy. In addition to the requirement for intravenous analgesics and parenteral nutrition, oral mucositis can lead to interruption of cancer therapy, and effective treatment is therefore a high priority in these patients. Trefoil factors, which are structural components of the mucus, have been shown to heal ruptures in the intestinal epithelium in mouse models of colitis. When these are provided prophylactically in a mouthwash containing a recombinant *L. lactis* strain, radiation-induced oral mucositis is significantly reduced in a hamster model of the disease. Human studies are currently underway, with promising results.

In addition to prophylactic treatment, genetically modified probiotics can also be used for therapeutic interventions. Experiments in murine models of ulcerative colitis have shown that administration of *L. lactis* expressing recombinant IL-10 downregulates ongoing inflammation. Preliminary clinical data suggest that similar results can be obtained in humans and that the treatment is well tolerated. Another example of the potential therapeutic use is in T1D. Preliminary evidence from recent-onset diabetic NOD mice suggests that combination therapy with *L. lactis* expressing IL-10 and pro-insulin allows effective reduction of the dose of anti-CD3 treatment, thereby limiting the potential toxicity associated with anti-CD3. The approach leads to substantial, sustained remission in this model of recent-onset diabetes.

A major challenge for these approaches is to achieve consistent dosing. Comparison of the numbers of live and total recombinant bacteria in faecal samples reveals a wide distribution among patients receiving set doses of the preparation. Consequently, the actual dose received by any given patient may be poorly controlled. When considering these bacteria as a delivery vehicle for a recombinant therapeutic, this is obviously an important limitation in interpreting the effects observed. It should be remembered, however, that this is also likely to be a consideration applicable to any probiotic. Thus, carefully controlled studies are required to identify the factors that determine viability and, therefore, dosage control in both genetically modified and naturally occurring probiotics.

Pharmaceutical regulatory requirements represent another major hurdle to be overcome in the development of probiotics, particularly genetically

modified strains. Good Manufacturing Practice must be complied with and, in addition to animal toxicology, studies must be undertaken to demonstrate identity, purity, stability, and potency. In the case of recombinant strains, pharmaceutical uses are considered to be deliberate release of a genetically modified organism into the environment. As such, comprehensive risk assessments and effective containment systems must be demonstrated. All of these factors are important public health considerations and are therefore a positive step rather than a hindrance. However, they place considerable demands upon the product development process that must be considered carefully.

Finally, an increasing area of interest is the role of prebiotics. Initial studies have explored the *in vitro* effects of galacto- and fructo-oligosaccharides on immune mechanisms and two primary prevention trials have been undertaken. Interestingly, some of the beneficial effects of human milk are related to the prebiotic oligosaccharide content. Combined provision of prebiotics and probiotics (known as synbiotics) now represent an interesting area for research into prevention strategies. Future studies will need to explore not only the effectiveness of clinical interventions but also the mechanism of action of these supplements.

Dietary modification

Evidence from multiple studies shows that breastfeeding is associated with a reduced risk of obesity later in life. Furthermore, the risk of obesity continues to decline with longer periods of breastfeeding, up to around 7 months. Comparison of breastfed and formula-fed infants in affluent populations shows that breastfeeding is also associated with slower weight gain. At the same time, high early weight gain carries a 2- to 3-fold greater risk of obesity at school age and in adulthood. Thus, the relationship between breastfeeding and early weight gain may play a role in determining the likelihood of childhood and adult obesity, which is a key risk factor for other chronic diseases.

The health-economic impact of controlling early weight gain is predicted to be enormous if we consider the relationship between obesity and chronic diseases such as type-2 diabetes and cardiovascular disease. Similar gains in terms of health expenditure and quality-adjusted life years can be expected from early interventions to prevent diseases such as asthma, allergy, and perhaps even T2D. A major challenge, then, for future research will be to determine the extent to which dietary modification can directly or indirectly influence the risk of developing these diseases. In many cases, this will involve

interplay between nutritional or environmental factors and the development of the microbiota. Consequently, it will be essential to integrate findings from epidemiological research with those from investigation of the microbiome in order to design appropriate intervention studies. Furthermore, mechanistic insights are urgently required to explain the biological links between the diseases. In the case of asthma, for instance, several mechanisms have been proposed to explain the link with obesity. These include a role for low-grade inflammation centred on the adipose tissue or an effect on lung development and function. No clear causal relationships have yet been demonstrated, however.

Taking into account the role of genetic susceptibility will be important in determining who is likely to benefit from specific interventions. The role of gene expression in determining susceptibility, disease course, and response to treatment or preventive measures may itself be modifiable, however. Epigenetic modifications such as methylation play a clear role in regulating gene expression and these variations can be controlled by a variety of extrinsic factors, including nutrition. Altering the levels of methyl donors in the maternal diet of Agouti mice – a classic animal model in which the level of CpG methylation in the agouti promoter regulates coat colour and other phenotypes such as obesity and susceptibility to diabetes and cancer – changes the phenotype of the offspring. Thus, epigenetic changes resulting from dietary modification can determine whether a specific genotype generates a phenotype with health-related consequences. But is there evidence that such modulation occurs in other diseases?

When experimentally induced asthma is analysed in the offspring of mice fed on a diet rich in methyl donors during pregnancy, the severity of airway disease is greater than in the offspring of animals fed on a diet low in methyl donors. Interestingly, this effect appears to be transgenerational, suggesting that maternal environmental exposures could influence the heritable risk of allergic airways disease. This observation in mice is supported by epidemiological studies on the effect of perinatal folate ingestion, which may be associated with a slight increase in the risk of wheeze and physician-diagnosed asthma in young children.

A great deal remains to be understood about the relationship between environmental inputs, epigenetic changes, and specific disease phenotypes before dietary modification can realistically be used to influence the epigenetic control of disease. Nevertheless, clinical research into the impact of dietary modification on chronic disease must take

into account epigenetic changes alongside genotype as a major factor likely to determine outcome. Clearly, then, future clinical developments will be dependent upon close collaboration between clinicians, epidemiologists, and molecular biologists, among others.

Tolerance induction

As with early dietary modification and the prophylactic use of natural or genetically modified probiotics, tolerance induction can be used as an approach to prevent the onset of allergic disease rather than treat existing disease. Studies in animal models have now shown that recombinant polyvalent chimeric allergens can be effectively used for the prophylactic treatment of allergy through a mechanism involving modulation of regulatory cytokines. Prophylaxis is found to be more effective than therapeutic intervention, while mucosal application appears to be more effective than systemic treatment.

Tolerance induction as a prophylactic measure follows a similar model to vaccination used in early childhood to protect against infectious diseases. The obvious advantage of such an approach is that, by preventing the onset of disease, it improves individual quality of life and reduces healthcare expenditure compared with curative measures. However, a number of points remain to be addressed before its widespread use can be recommended. These include the appropriate timing of prophylaxis, the duration of protection, and the target groups (in all infants or only in those identified as having an increased risk).

Dietary modification can also be used to induce tolerance in infants at risk for allergic disease. When children with a family history of atopy receive hypoallergenic, hydrolysed protein formula instead of cow's milk formula, the risk of allergies and atopic dermatitis is significantly reduced. However, the effectiveness of this approach differs according to the type of hypoallergenic formula. Furthermore, there are a number of safety issues that remain to be resolved.

Looking Forward – the Grand Challenges

Significant progress has been made in our understanding of the mechanisms underlying chronic inflammatory disease. The complex interactions between host, environment, and microbiome that ultimately determine the transition to chronic, pathological inflammation, however, place particu-

lar demands upon clinical research to identify new therapies and preventive measures.

Improved phenotyping

The design and interpretation of clinical trials is critically dependent upon patient inclusion criteria. Since evidence increasingly suggests that current disease classifications are inadequate, it is likely that treatment groups may not always be phenotypically homogeneous. As a result, the outcome of clinical trials may not accurately reflect the efficacy of a drug in the appropriate target population. An important challenge, then, for future clinical research will be to enhance the phenotypic resolution of existing disease classifications.

Promising results in animal models can also fail to yield the expected outcome in human studies. As we improve our classification of disease phenotypes and subphenotypes in humans, it will be important to use this information to inform the choice of animal models used to identify therapeutic targets and screen potential drugs. Thus, improving the correspondence between human phenotypes, animal models, and patient selection criteria in clinical trials should increase the likelihood of success in clinical research and drug development.

Effective translational research

The hypothesis-generating research characterised by genome- and metagenome-wide association studies yields large amounts of data that must be validated in hypothesis-testing, experimental models. A key challenge for the future, then, will be to obtain maximum benefit from these approaches and increase the likelihood of identifying effective treatment and prevention strategies. This calls for unbiased, high-throughput translational research in which the right targets are analysed in the most appropriate model and potential treatments explored in the right patients. In practice, this is a logistic rather than a technological or intellectual challenge. It is nevertheless a formidable task. For a number of years, there has been an increasingly rigid separation between academic and pharmaceutical industry research. Furthermore, despite the bioscience industry having a clear interest in promoting omic approaches, access to the necessary technology and regulations on the exploitation of the data generated present practical and ethical barriers to progress that need to be overcome. Given the size of the task and the economic implications, strong academic-industrial partnerships that take into account the needs of society will need to be established. This is essential if we are to advance our understanding of complex inflammatory diseases and benefit European healthcare.

Health economics

As research provides insights into the intrinsic mechanisms, environmental triggers, and protective factors that influence the development of chronic inflammatory disease, both preventive and therapeutic strategies can begin to be considered. A major challenge for future health-economic analyses will be to determine the effect of nutritional modification for the prevention and treatment of disease. Furthermore, health investment will need to take into account not only the treatment of existing disease that has its causes in infancy and childhood but also the prevention of disease in future adult populations.

References

1. Holgate, S.T. A look at the pathogenesis of asthma: the need for a change in direction. *Discov. Med.* **9**, 439–447 (2010).
2. Johannsen, H. & Prescott, S.L. Practical prebiotics, probiotics and synbiotics for allergists: how useful are they? *Clin. Exp. Allergy* **39**, 1801–1814 (2009).
3. Kopp, M.V. & Salfeld, P. Probiotics and prevention of allergic disease. *Curr. Opin. Clin. Nutr. Metab. Care* **12**, 298–303 (2009).
4. Salminen, S. *et al.* Functional food science and gastrointestinal physiology and function. *Br. J. Nutr.* **80** Suppl 1, S147–S171 (1998).
5. Rottiers, P., De Smedt, T. & Steidler, L. Modulation of gut-associated lymphoid tissue functions with genetically modified *Lactococcus lactis*. *Int. Rev. Immunol.* **28**, 465–486 (2009).
6. *Advances in Experimental Medicine and Biology* Vol. 646 (eds. Koletzko, B., Decsi, T., Molnár, D. & Huntly, A.) (Springer Science + Business Media, New York, 2009).
7. Koletzko, B. *et al.* Lower protein in infant formula is associated with lower weight up to age 2 y: a randomized clinical trial. *Am. J. Clin. Nutr.* **89**, 1836–1845 (2009).
8. Brandtzaeg, P. Food allergy: separating the science from the mythology. *Nat. Rev Gastroenterol. Hepatol.* **7**, 380–400 (2010).

Key points

To translate research findings into treatment and prevention strategies that will benefit European healthcare, substantial efforts are now required to:

- Improve treatment options through the use of patient selection criteria based on deep phenotyping (clinical and biological)
- Enhance patient stratification through the identification of novel biological markers
- Develop effective primary prevention strategies that take into account the limited success of probiotics achieved to date and explore the potential of prebiotic approaches
- Explore the clinical potential of tolerance induction via safe microbial compounds and elucidate the mechanistic justification for their use

5.

Strategic Recommendations



At the end of each workshop in Barcelona, delegates discussed strategic recommendations that they felt were relevant to the future of research into chronic disease. These were organised into six broad categories (Figure 5, p. 30).

Future Scenarios for Research into Chronic Inflammatory Disease

The future direction of research into chronic inflammatory diseases depends not only on the identification of the grand challenges within the field but also on the factors that impact upon our ability to address them. As part of a scenario-building exercise designed to model the future of research into chronic disease, seven different influencing fields were identified (Figure 3).



Figure 3. Influencing fields impacting upon future research into chronic inflammatory disease.

Each field encompasses key considerations that will shape the future of research into chronic disease. The type of research that can be undertaken and the outcomes that can reasonably be expected are obviously conditioned by the infrastructure that supports it and the availability of the required expertise. Establishment of interdisciplinary research, for instance, requires not only the provision of appropriate infrastructure but also the training of scientists in the analysis of complex datasets and in cross-disciplinary communication. These foundations cannot be laid, of course, without adequate financial support. A key consideration for the future direction of research, then, is whether sufficient resources can be made available to fund a broad range of topics or whether research must focus only on a limited subset of questions? One determining factor in this question is the role of industry and its relationship with academia. Will industry, for instance, play an important role in determining the research agenda and to what extent will this be influenced by science policy and academic research strategy? Furthermore, although science policy can be assumed to be influenced by the needs of society, strategic decisions will also determine the level of public sympathy to the needs of research and the role of stakeholders in the provision of healthcare. Public support will in turn affect the type of research that can be undertaken, particularly considering ethical issues such as access to sensitive data and use of biological samples.

Taking all of these and other considerations into account, the seven fields identified as influencing the future of research into chronic disease could be subdivided into 15 influencing factors (Figure 4). When these different influencing factors are explored in a variety of possible future scenarios, we can begin to predict the impact of current decisions.

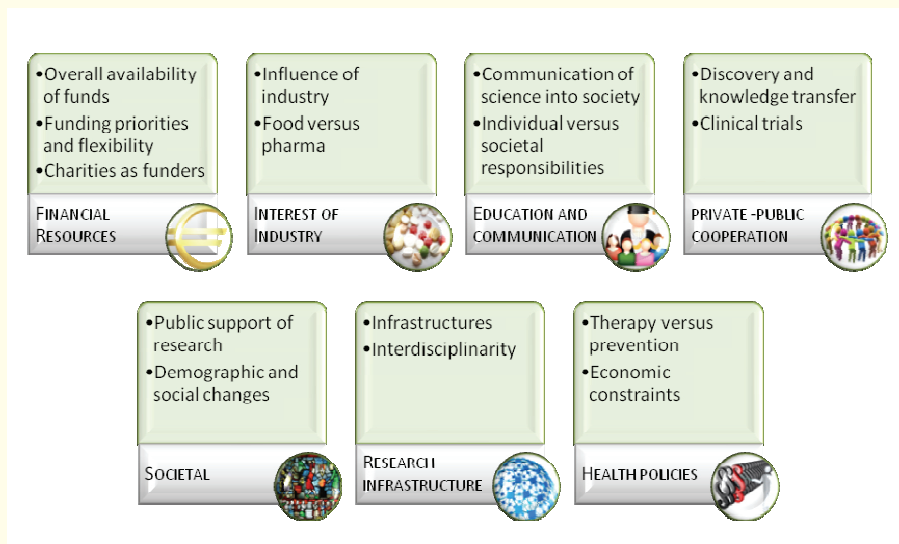
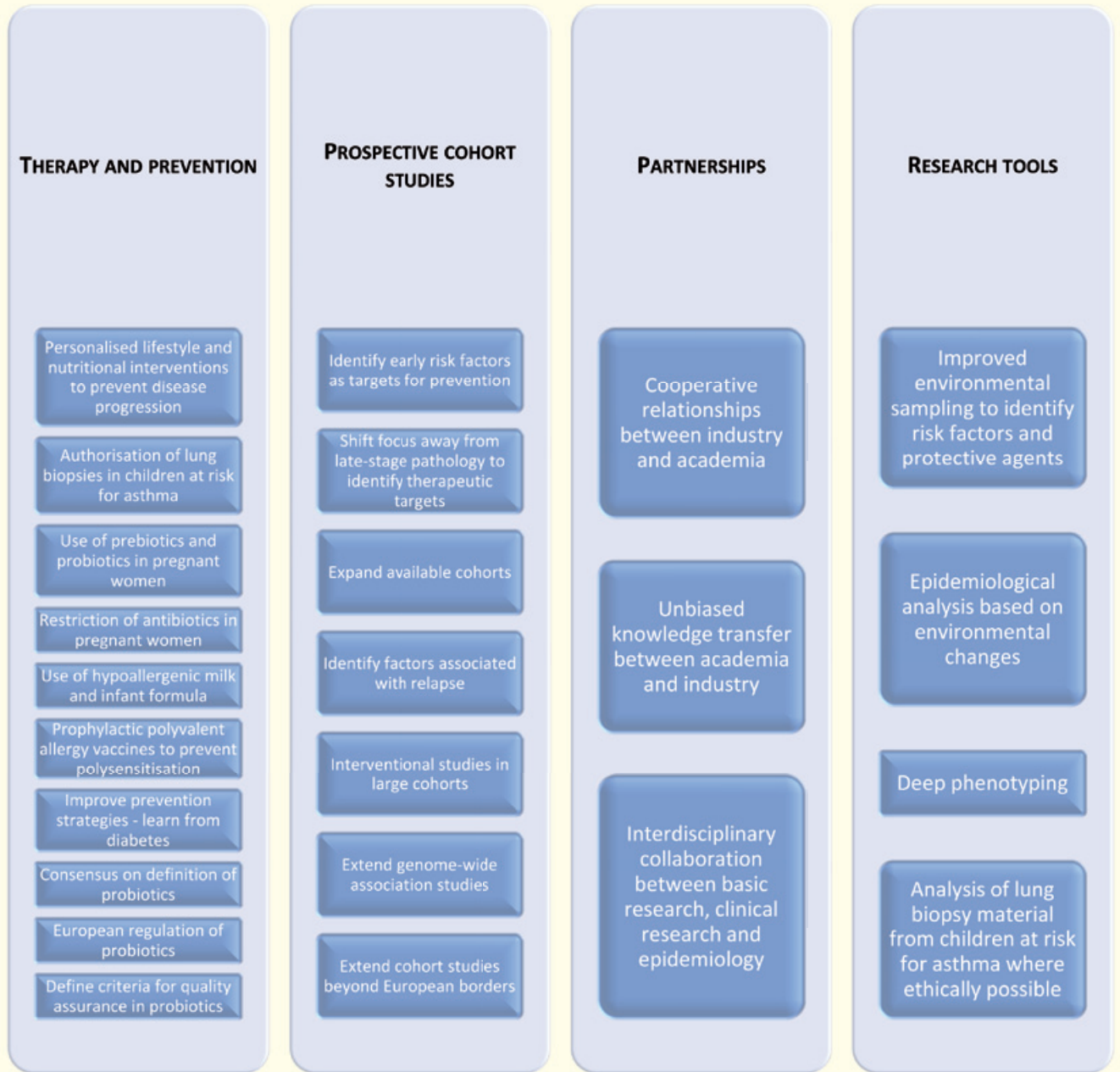


Figure 4. Factors influencing research into chronic inflammatory disease.

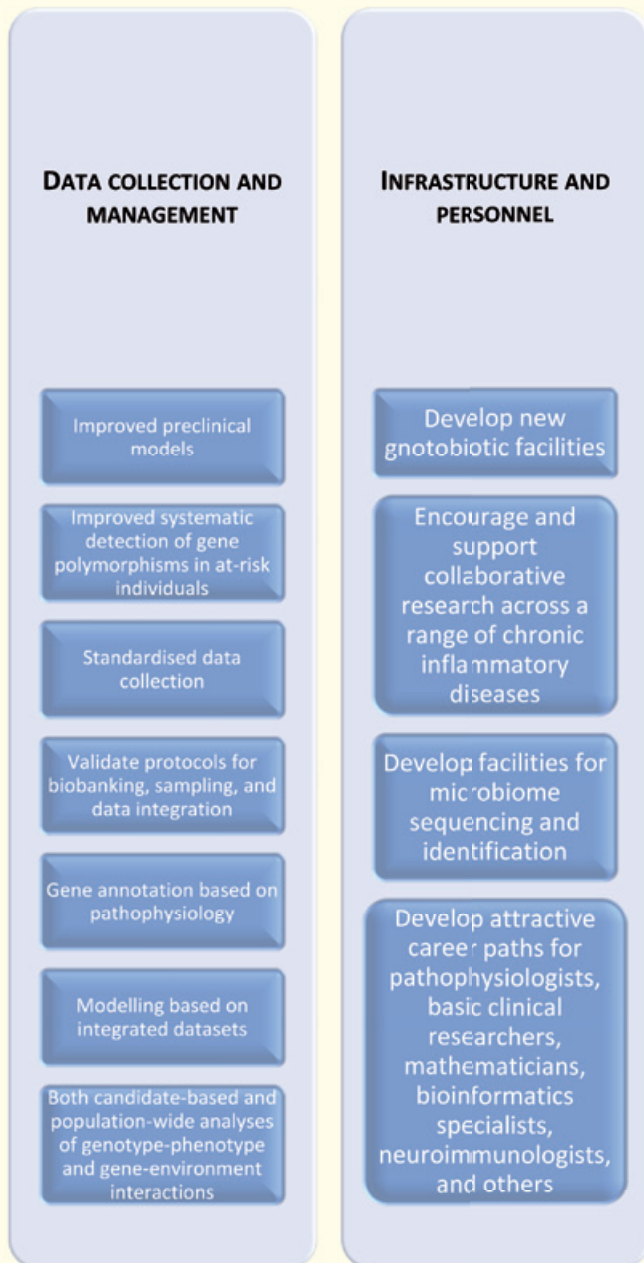


Figure 5. Strategic Recommendations for future research into chronic inflammatory disease

benefits arising from this knowledge generation. The failure to exploit the commercial potential of scientific knowledge may lead to a weakening of Europe's position in the healthcare industry and even a danger that European research investment is exploited by companies in other parts of the world. The limited economic return generated from substantial investment in infrastructure and long-term research projects could then ultimately threaten future investment.

In an alternative scenario, European competitiveness in the healthcare industry is ensured through investment in goal-directed research driven by interaction between industry and academia in those areas expected to generate marketable products. The resulting emphasis, however, could be predicted to largely ignore prevention. Marketing of products designed to alleviate symptoms might also fuel public scepticism over the relationship between industry and policy. As a consequence, public sympathy for the needs of research into chronic diseases is expected to be reduced and there will be an emphasis on data protection and adherence to ethical standards. Under these conditions, the influence of independent researchers is extremely limited. The long-term consequences of such a scenario could be a relative failure to advance understanding of causative factors and a paucity of public health measures to improve population wellbeing and reduce healthcare expenditure.

Optimising the Relationship Between Academia and Industry

If adequate infrastructure and research funding is generated along with public cooperation in the development of biobanks and large-scale longitudinal studies, significant insights are predicted to be gained into the pathophysiology of chronic inflammatory diseases. Knowledge generation is assumed to be optimal, resulting in widespread identification of risk factors and therapeutic targets. However, without appropriate mechanisms to ensure knowledge transfer between academia and industry, healthcare delivery is likely to suffer from a lack of innovation and limited economic

Balancing Health-Economic Considerations

Concerns over spiralling healthcare costs could have multiple effects on health policy. Firstly, emphasising the role of lifestyle and societal factors in chronic disease could lead to a focus on prevention. Under these conditions, substantial resources would be invested in public health education, while the availability of funding for research would be reduced. Publicly funded research, as well as research in industry, would be focused almost exclusively on prevention. Research in other areas would therefore be dependent upon support from charities and would not have access to the infrastructure available for prevention research. Most of the prevention strategies would address the most prevalent chronic diseases and, in the absence of well-funded basic

research, the approaches would be reliant on current scientific knowledge.

In an alternative scenario, the need to control healthcare expenditure results in a requirement for proven cost-effectiveness at all levels. Under these conditions, there would be limited investment in research infrastructure and basic research funding would be restricted by the difficulty of demonstrating a reliable return on investment. Support for investigator-driven clinical trials aimed at identifying the most cost-effective therapies would be expected to grow while approval of new drugs would be increasingly challenging. As a consequence, the pharmaceutical industry would be expected to focus most of its budget on incremental innovation and reduce its support for long-term basic research with the potential for groundbreaking innovation. The overall effect of these policy decisions would thus be to substantially reduce the potential for innovation and to slow the rate of progress in healthcare development.

6.

Recommendations for Research into Gene-Environment Interactions in Chronic Disease



During the consensus conference in Berlin, delegates discussed the grand challenges and strategic targets identified during the Barcelona workshops in light of the insights gained from the scenario-building exercise. The outcome is the following series of considerations for the future of research into gene-environment interactions in chronic disease.

Therapy and Prevention

In order to prioritise recommendations, we must establish the intended outcome of research into chronic inflammatory disease. Thus, if the ultimate goal is to reduce the burden of these diseases, research priorities must support the development of strategies for disease management. Such strategies, however, can be separated into two fundamentally different categories – therapy and prevention – and recommendations should make clear distinctions between them. Clinical development must take into account core differences in the regulatory requirements for therapeutic and preventive interventions. Research strategies and models for collaboration between different stakeholders must also bear in mind the different product-development and marketing considerations affecting the pharmaceutical, diagnostics, and food industries. In order to provide a clear scientific basis for the clinical development of novel therapies and prevention strategies, basic research will also need to distinguish between disease initiation, maintenance, and exacerbation.

For both therapy and prevention a stratified approach needs to be developed. Stratified therapies will be applied based on new disease clas-

sifications, which, in turn, are defined by clinical and biological (sub-) phenotypes. This approach requires deep environmental, clinical, and biological phenotyping of patients. This must go hand in hand with the identification and development of novel biomarkers, providing an easy in-vitro diagnostic approach for phenotypic classification of the patients. An important approach towards this goal will be the integration of omics data, requiring huge investments in bioinformatics and systems biology. Significant progress can be only achieved by the development of an advanced level of academic-industrial partnerships involving both the pharmaceutical in-vitro diagnostics industries.

Key issues for prevention include the selection of appropriate populations and the long-term tolerability of putative protective agents. In the current climate, the term probiotic should be employed with caution and substantial efforts will be required to develop a more rigorous scientific evidence base supporting their use in chronic inflammatory disease. It may therefore be advisable to develop strategies to improve the characterisation of any microbial preparation intended to protect against the initiation or progression of disease. This should include a comprehensive description of its mechanism of action. Based on current knowledge, a clear separation must be made between research into probiotic effects and that addressing the nature and influence of the microbiota. An important target for future clinical research will nevertheless be the therapeutic manipulation of resident microbial communities. It will therefore be important to define the regulatory considerations that will influence such developments.

Grouping patients together without paying adequate attention to possible subphenotypes may lead to a failure to recognise or approve effective treatments. Without this sort of deep phenotyping, it will be impossible to determine whether a potential therapy is clinically ineffective or simply inappropriately targeted. Secondly, the experimental models used to identify putative drug targets must be sufficiently well characterised to determine whether they adequately effect the human disease. Thus, both patient selection criteria and choice of animal model will be dependent upon improving our classification of specific disease phenotypes. This calls for unbiased, high-throughput translational research in which the right targets are analysed in the most appropriate model and potential treatments explored in the right patients.

Large Cohort Studies

Large prospective cohort studies are absolutely central to any effort to understand the factors responsible for the initiation, maintenance, and exacerbation of chronic inflammatory disease. Such studies should, wherever possible, be initiated prior to birth in order to take into account the impact of early life. In addition, they should include collection of a wide range of relevant biological samples, including biopsy material where ethically possible, characterisation of the microbiome, and collection of epigenomic data. The biological systems analysed should be as extensive as possible and take into account fields such as neuroscience and psychosocial variables such as stress. Furthermore, sampling must ensure the availability of data not only on genomic, clinical and environmental factors but also epigenomic, metabolic, nutritional, microbial, and other key variables thought to influence the pathophysiology of chronic disease. The complexities of this task may necessitate the preparation of explicit contracts for use with patients who participate in these studies, including permission to obtain tissue biopsies and other biological samples from children. Furthermore, to take account of new insights obtained over the course of long-term cohort studies, it will be important to make provisions for retrospective access to study participants. A key concern in such a large-scale approach will be to ensure international collaboration and coverage of populations with different lifestyles and environmental exposures. Specific issues, such as the effect of vaginal versus cesarian delivery, should also be addressed within ongoing cohorts.

The need for deep phenotyping across the life course is increasingly apparent, given the substantial gaps in our understanding of normal physiology and the phenotypes associated with the transition to late-stage disease. In-depth characterisation of subsets of individuals in the context of a larger, well-characterised cohort is recognised to provide much greater opportunity for the meaningful extrapolation of findings and is expected to strengthen the evidence base for clinical research. The use of embedded case-control studies is also predicted to generate key insights into factors that may protect against disease. In order to ensure the continued value of large cohorts, however, it will be important to prevent the introduction of bias caused by reducing the running time of the cohort or by restricting ongoing analysis to specific populations.

We recognise the importance of ensuring access to datasets from large prospective cohorts. Researchers with expertise in particular areas should be allowed to address questions in clearly defined subpopulations while maintaining the integrity of the larger cohort. Such considerations are complicated by the potentially conflicting interests of the different stakeholders who provide funding and support. Efforts will therefore be required to achieve consensus on access to data from large cohorts that may receive both public and private funding.

Partnerships

The shifting global pattern of chronic disease indicates that we currently have unrivalled opportunities to identify key factors conferring both risk and protection. In order to take advantage of these opportunities, it will be essential to collaborate with countries in which the incidence of these diseases is increasing rapidly. This will require that future policy on research into chronic inflammatory diseases maintain a global focus and foster strong collaborations with developing countries. Research projects should be established in regions with low or developing risk of chronic inflammatory disease, particularly those countries in which the incidence of disease is known to be changing. Furthermore, the establishment of parallel birth cohorts in low- and high-risk regions can be considered an urgent priority.

In addition to global collaborations, cross-disciplinary partnerships will be essential for the future of research into chronic inflammatory disease. Such partnerships should extend beyond traditional disciplines such as epidemiology, clinical research,

and microbiology, and should ensure that valuable insights can be obtained from fields such as mathematics, virology, and ecology. Furthermore, the common themes that are currently being identified in the pathophysiology of a range of different diseases present a fantastic opportunity for cross-fertilisation given the right conditions. In order to exploit these opportunities, then, the establishment of knowledge networks should be made a priority as a driving force for interdisciplinary research.

Another key driving force for future research into chronic disease will be the establishment of effective private-public partnerships characterised by greater fluidity in the exchange of information between academia and industry. To achieve this, agreements should be made on issues such as access to data and ownership of results. Such agreements should ensure the maintenance and stability of long-term partnerships that recognise the needs of all stakeholders. Furthermore, they will need to establish appropriate ethical frameworks for data sharing.

Research Tools

Experimental models play a crucial role in establishing the mechanistic basis of disease and as tools for the screening of potential therapeutic and preventive strategies. However, it is apparent that existing models may be inadequate to address many of the questions now facing research into chronic inflammatory disease. The effectiveness of future research will therefore be critically dependent upon the development of improved models for use in experimental and translational research. In addition to animal models, human organ-based systems are urgently required as a potential stepping stone for the screening of candidate interventions prior to human testing.

Consensus is urgently needed on how to obtain suitable models in which to analyse the mechanisms of chronic inflammatory disease. Current animal models based on single gene changes may be inadequate to address the complexity of multi-gene effects. Similarly, animal housing conditions optimised to reduce experimental variability (i.e. defined commensal flora, absence of pathogens and defined diet) present problems for research aimed at understanding the environmentally modulated course of disease. Consequently, new research strategies are required that take the diversity of the microbiome into consideration in the choice of experimental models and the potential reproducibility of the results.

Gnotobiotic models will be key tools with which to improve our understanding of the role of host-microbial interactions in chronic inflammatory disease. However, their use is currently limited by the availability of methods for the culture and characterisation of microbial populations. Consequently, efforts must be made to increase the availability of cultivable isolates. In addition, the paucity of microbiological tools restricts our ability to understand the development and dynamics of microbial ecosystems such as those found in the gut or respiratory tract and more research is required to assess the factors that influence microbial ecology in healthy and diseased hosts.

Our capacity to identify those external factors that trigger or protect against disease is determined by the extent to which we can accurately sample them. Currently, our ability to clearly define and characterise the nutritional input to the organism and how that input is processed and finally removed from the system is extremely limited. Likewise, we only have a small number of tools at our disposal with which to sample the environment as it impacts upon the human organism, thereby restricting substantially the power of epidemiological analyses. Future research will thus require new tools with which to characterise environmental and nutritional factors.

Data Generation and Management

The results obtained in experimental models of inflammatory disease can vary from one group to another with no clear explanation for the difference. Potential contributing factors include variation in the microbiota between animals reared in different facilities and also nutritional and metabolic differences resulting from variation in chow diets. All of these factors need to be more carefully characterised and harmonised in order to ensure that research is rigorously controlled and the results more easily interpreted. Since similar considerations apply to other types of study, a priority for future research will be the harmonisation of protocols.

Dealing with complex research questions is dependent upon incremental increases in knowledge. As a consequence, data from long-term cohort studies must be continually reassessed in the same population as new insights are gained into disease mechanisms. It is therefore of critical importance that sample collection, classification, and storage are managed in such a way as to ensure that the next level of complexity can be addressed when the time comes. This means harmonisation of data-

collection, transport and storage protocols, and development of standardised systems for cataloguing and accessing datasets. Importantly, substantial investment will be required in developing the bioinformatics and systems biology approaches required for the analysis of the datasets generated. While this is a general requirement in many areas of biomedical research, the additional complexity introduced by the microbiome makes it particularly urgent for research into chronic inflammatory disease.

Infrastructure and Personnel

The type of interdisciplinary research that will be required to achieve meaningful insights into the causes, treatment and prevention of chronic inflammatory disease will necessitate the development of specific infrastructure. Firstly, research consortia will need to be organised in such a way as to allow regular face-to-face interaction among researchers. In addition, the physical infrastructure will need to ensure efficient access to core services such as gnotobiology facilities, next-generation sequencing and bioinformatics support. Building the electronic infrastructure to allow integrated approaches involving open collaboration between researchers has the potential to bring about the step change in thinking that may be crucial in driving future research.

Funding of interdisciplinary research consortia will need to take into consideration the particular needs of this type of research. First and foremost, efforts should be made to support unbiased approaches. To achieve this, funding decisions should be made by expert panels in which no specific discipline is overrepresented. Secondly, the conditions required for effective interdisciplinary research are incompatible with a funding structure in which support for this type of research is in direct competition with that available for its component fields. This means that dedicated funding should be provided for interdisciplinary research into chronic inflammatory disease. Finally, bringing together disciplines such as epidemiology and mechanistic research in animal models inevitably requires substantial time investment in order to develop fruitful working relationships. Such a requirement is incompatible with competitive short-term funding cycles. A long-term approach toward funding of interdisciplinary research is therefore required.

In order to ensure effective cross-disciplinary communication, substantial investment must be made in the training and education of young researchers. A new generation of biological and

medical scientists will need to be ready to exploit rapid developments in information technology and use insights not only from a range of scientific disciplines but also fields as diverse as finance and engineering. An effective way to achieve this will be to establish international graduate schools that provide specific training in interdisciplinary biomedical research. Furthermore, career structures should be established that ensure the long-term attractiveness and stability of interdisciplinary research for young scientists. Criteria for career success, such as publication record based on competitive authorship models, may need to be reconsidered.

Consensus recommendations

Based on these considerations, the delegates in Berlin identified the following ten key areas as having the highest priority for research into chronic inflammatory disease:

1. Research should distinguish clearly between therapy and prevention
2. Large prospective cohort studies including deep phenotyping should be made a priority
3. Strategies and tools should be developed to ensure adequate sampling of the environment
4. A global (international) approach should be taken to understanding chronic inflammatory disease
5. Effective interdisciplinary research strategies must be established
6. New tools and experimental models must be developed
7. Protocols for data collection, handling, and storage need to be harmonised
8. Substantial investment must be made in infrastructure, personnel, and development of research tools
9. Dedicated funding must be provided for interdisciplinary research
10. Effective public-private partnerships must be developed to ensure free exchange of information

Annex

Committee members, Speakers and Participants

Committee Members

Scientific Committee

Chairs:

- Professor Dirk Haller
Technical University Munich, Germany
- Professor Harald Renz
Philipps-University Marburg, Germany

Members:

- Professor Ingo B. Autenrieth
University Hospital Tübingen, Germany
- Professor Per Brandtzaeg
Oslo University Hospital, Norway
- Professor William O. Cookson
Imperial College London, United Kingdom
- Professor Thomas T. MacDonald
Barts and the London School of Medicine and Dentistry, United Kingdom
- Professor Erika von Mutius
Dr. von Hauner Children's Hospital, Germany
- Professor Rudolf Valenta
Medical University of Vienna, Austria

Management Committee

Chair:

- Professor Martin Röllinghoff
EMRC Core Group member, Erlangen-Nuremberg University, Germany

Members:

- Dr Marc Heppener
ESF Director of Science and Strategy Development, Strasbourg, France
- Professor Stephen T. Holgate
Southampton General Hospital, United Kingdom
- Professor Liselotte Højgaard
EMRC Chair, Rigshospitalet, University of Copenhagen, Denmark
- Professor Andrew MacPherson
Inselspital, University of Bern, Switzerland

Strategic Workshops, 19-22 October 2010, Barcelona (ES)

Workshop 1: Intrinsic Mechanisms in Chronic Disease

Chair:

- Professor Dirk Haller
Technical University Munich, Germany

Co-Chair:

- Professor Per Brandtzaeg
Oslo University Hospital, Norway

Speakers:

- Professor John Bienenstock
The Brain-Body Institute, St. Joseph's Healthcare Hamilton, Canada
- Professor Stephan Bischoff
Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany
- Professor Richard S. Blumberg
Brigham and Women's Hospital, Harvard Medical School, Boston, United States
- Dr Jayne Danska
The Hospital for Sick Children, Toronto, Canada
- Professor Andreas Diefenbach
Institute of Medical Microbiology & Hygiene, University of Freiburg, Germany
- Professor Nicola L. Harris
École Polytechnique Fédérale de Lausanne, Switzerland
- Dr Bastiaan T. Heijmans
Leiden University Medical Centre, The Netherlands
- Professor Mathias Hornef
Institute for Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Germany
- Professor Jean-Pierre Hugot
Hôpital Robert Debré, INSERM U843, Paris, France
- Professor Hans-Georg Joost
German Institute of Human Nutrition (DIfE), Potsdam-Rehbrücke, Germany
- Professor Francine Kauffmann
Epidemiology and Statistics, INSERM, Villejuif, France

- Professor Martinus Lovik
National Institute of Public Health (NIPH Folkehelse), Oslo, Norway
- Professor Michael Müller
Division of Human Nutrition, Wageningen University, The Netherlands
- Professor Oliver Pabst
Institute of Immunology, Hannover Medical School, Germany
- Professor Manolis Pasparakis
Institute for Genetics, University of Cologne, Germany
- Professor Lesley E Smythies
University of Alabama, Birmingham, United States
- Dr Emily Swindle
Faculty of Medicine and Health and Life Sciences, University of Southampton, United Kingdom
- Professor Hartmut Wekerle
Max-Planck-Institute of Neurobiology, Martinsried, Germany
- Dr Ola Winqvist
Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden

Workshop 2: Extrinsic Mechanisms in Chronic Disease

Chair:

- Professor Erika von Mutius
Dr. von Hauner Children's Hospital, Germany

Co-Chair:

- Professor Ingo B. Autenrieth
University Hospital Tübingen, Germany

Speakers:

- Dr Heiner Boeing
German Institute of Human Nutrition (DIfE), Berghol-Rehbrücke, Germany
- Professor William O. Cookson
Imperial College London, United Kingdom
- Dr Dusko Ehrlich
Microbiology and Food Chain (MICA), INRA, Jouy-en-Josas, France

- Professor Harry Flint
Rowett Institute of Nutrition and Health, University of Aberdeen, United Kingdom
- Dr Valerie Gaboriau-Routhiau
INRA-INSERM, U910, Jouy-en-Josas, France
- Dr Dick Heederik
Institute of Risk Assessment Sciences, University of Utrecht, The Netherlands
- Professor Denise Kelly
Rowett Institute of Nutrition and Health, University of Aberdeen, United Kingdom
- Professor Michiel Kleerebezem
NIZO Food Research, Wageningen University, The Netherlands
- Professor Rob Knight
University of Colorado at Boulder, United States
- Professor Bart Lambrecht
Faculty of Medicine and Health Sciences, Ghent University, Belgium
- Professor Seppo Salminen
Functional Foods Forum, University of Turku, Finland
- Professor Stefan Schreiber
Institute for Clinical Molecular Biology, Christian-Albrechts-University, Kiel, Germany
- Professor Helena Tlaskalova-Hogenova
Institute of Microbiology of the Academy of Sciences of the Czech Republic, Prague, Czech Republic
- Professor Jerry M Wells
Faculty of Animal Sciences, University of Wageningen, The Netherlands
- Dr Agnes Wold
Institute of Biomedicine, University of Gothenburg, Sweden

Workshop 3: Therapy and Prevention – Emerging Concepts

Chair:

- Professor Harald Renz
Philipps-University Marburg, Germany

Co-Chair:

- Professor William O. Cookson
Imperial College London, United Kingdom

Speakers:

- Professor Hans Bisgaard
Faculty of Health Science, Copenhagen University, Denmark
- Dr Charlotte Braun-Fahrländer
Institute of Social and Preventive Medicine, University of Basel, Switzerland
- Professor Graham Devereux
Applied Health Sciences, University of Aberdeen, United Kingdom
- Dr Francisco Guarner
University Hospital Vall d'Hebron, Barcelona, Spain
- Professor Stephen Holgate
Southampton General Hospital, United Kingdom
- Dr John Hollingsworth
Duke University Medical Centre, Durham, United States
- Professor Berthold Koletzko
Dr. von Hauner Children's Hospital, Ludwig-Maximilians University, Munich, Germany
- Professor Sibylle Koletzko
Dr. von Hauner Children's Hospital, Ludwig-Maximilians University, Munich, Germany
- Dr Markus Neurath
Department of Medicine, University of Erlangen-Nürnberg, Germany
- Professor Seppo Salminen
Functional Foods Forum, University of Turku, Finland
- Professor Jürgen Schwarze
Queens Medical Research Institute, University of Edinburgh, United Kingdom
- Dr Lothar Steidler
ActoGeniX NV, Zwijnaarde, Belgium

- Professor Joerg Vogel
Institute of Molecular Infection Biology, University of Würzburg, Germany
- Dr Markus Weckmann
Klinik für Kinder- und Jugendmedizin, Universitätsklinikum Schleswig-Holstein, Germany
- Professor Ursula Wiedermann
Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Austria

Participants:

- Dr Stephane Berghmans
European Science Foundation, Strasbourg, France
- Dr Julia Frick
Universitätsklinikum Tübingen, Germany
- Dr Olive Leavy
Senior Editor, Nature Reviews Immunology, London, United Kingdom
- Dr Georg Munz
Observer to EMRC Core Group, Deutsche Forschungsgemeinschaft, Bonn, Germany
- Dr Iain Patten
Medical writer, Valencia, Spain
- Dr Nathalie Spielwoy
European Science Foundation, Strasbourg, France
- Professor Raivo Uibo
EMRC Standing Committee Member, University of Tartu, Estonia

Consensus Conference, 15-16 March 2011, Berlin (DE)

Speakers:

- Professor Ingo B. Autenrieth
*University Hospital Tübingen,
Germany*
- Professor Per Brandtzaeg
*Rikshospitalet, University of Oslo,
Norway*
- Professor William Cookson
*Imperial College London,
United Kingdom*
- Professor Dirk Haller
*Technical University Munich,
Germany*
- Professor Stephen T. Holgate
*Southampton General Hospital,
United Kingdom*
- Professor Erika von Mutius
*Dr. von Hauner Children's Hospital,
Ludwig Maximilian University
Munich, Germany*
- Professor Harald Renz
*University Hospital Marburg,
Germany*

Participants:

- Dr Ruud Albers
Unilever R&D, The Netherlands
- Professor John Bienenstock
*St. Joseph's Healthcare Hamilton,
Canada*
- Professor Bengt Björkstén
Karolinska Institute, Sweden
- Professor Hervé Blottière
*INRA – National Institute
of Agronomic Research, France*
- Dr Stephanie Blum
Nestlé Health Science, Switzerland
- Professor Richard S. Blumberg
*Brigham and Women's Hospital,
Harvard Medical School,
United States*
- Professor Angela Brand
*Maastricht University,
The Netherlands*
- Dr Carmen Diaconu
*Romanian Academy of Sciences,
Romania*
- Dr Marta Fichna
Polish Academy of Sciences, Poland
- Professor Pierre Gianello
*Fonds de la Recherche Scientifique
(FRS), Belgium*

- Professor Tari Haahtela
*Helsinki University Central
Hospital, Finland*
- Dr Phillip Hahn
Helmholtz Association, Germany
- Dr Udo Herz
*Mead Johnson Nutrition,
The Netherlands*
- Professor Rolf Hultcrantz
*Karolinska University Hospital,
Sweden*
- Professor Erika Isolauri
*Turku University Central Hospital,
Finland*
- Professor Denise Kelly
*Rowett Institute of Nutrition and
Health, University of Aberdeen,
United Kingdom*
- Dr Lars Kristiansen
*European Science Foundation,
France*
- Dr Emmanuelle Maguin
*INRA – National Institute
of Agronomic Research, France*
- Professor Santos Mañes
*Spanish National Research Council
(CSIC), Spain*
- Professor Miriam Moffatt
*Imperial College London,
United Kingdom*
- Professor Michael Müller
*Wageningen University,
The Netherlands*
- Dr Georg Munz
*Deutsche Forschungsgemeinschaft,
Germany*
- Professor Mustafa Ozen
Istanbul University, Turkey
- Professor Isabel Palmeirim
*Foundation for Science and
Technology, Portugal*
- Dr Iain Patten
Medical writer, Spain
- Professor Jean-Claude Piffaretti
*Federation of European
Microbiological Societies,
Switzerland*
- Professor Martin Röllinghoff
*EMRC Core Group member,
Erlangen-Nuremberg University,
Germany*
- Professor Giovina Ruberti
National Research Council, Italy
- Professor Seppo Salminen
University of Turku, Finland
- Professor Nikolaos Siafakas
*European Respiratory Society,
Switzerland*
- Dr Lothar Steidler
ActoGeniX NV, Belgium
- Ms Nicole Stirnberg
*European Science Foundation,
France*
- Professor Mimi Tang
*Royal Children's Hospital
Melbourne, Australia*
- Dr Andreas Trepte
Max-Planck-Gesellschaft, Germany
- Professor Matthias Tschoep
*University of Cincinnati,
United States*
- Dr Birgit Wetterauer
*Federal Ministry for Education and
Research, Germany*



European Science Foundation
1 quai Lezay-Marnésia • BP 90015
67080 Strasbourg cedex • France
Tel: +33 (0)3 88 76 71 00
Fax: +33 (0)3 88 37 05 32
www.esf.org

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