CONGENIC MICE: CUTTING TOOLS FOR COMPLEX IMMUNE DISORDERS

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Autoimmune diseases are, in general, under complex genetic control and subject to strong interactions between genetics and the environment. Greater knowledge of the underlying genetics will provide immunologists with a framework for study of the immune dysregulation that occurs in such diseases. Ascertaining the number of genes that are involved and their characterization have, however, proven to be difficult. Improved methods of genetic analysis and the availability of a draft sequence of the complete mouse genome have markedly improved the outlook for such research, and they have emphasized the advantages of mice as a model system. In this review, we provide an overview of the genetic analysis of autoimmune diseases and of the crucial role of congenic and consomic mouse strains in such research.

INBRED STRAIN
A strain that is essentially homozygous at all genetic loci. In mice, such strains are produced by brother–sister mating for at least 12 sequential generations, or more if other breeding systems are used.

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Over the past twenty years, there has been rapid progress in identifying the genes that are involved in many congenital diseases (Online Mendelian Inheritance in Man, OMIM; BOX 1). Progress has almost exclusively involved diseases that are under monogenic (single-gene) control, such as Bruton's autoimmune syndrome and X-linked agammaglobulinaemia[1], and so far, more than 1,000 such Mendelian disease genes have been identified. Other than infectious diseases, however, the most important public-health challenges in terms of the number of individuals that are affected are diseases that are under multi-factorial and multigenic or polygenic control. Resulting from complex and often subtle interactions between genetic and non-genetic factors, these diseases are an important cause of morbidity and mortality in the adult population. Examples include arteriosclerosis and hypertension, and several diseases in which the immune system has a determinant role, for example insulin-dependent diabetes mellitus (IDDM), rheumatoid arthritis, asthma, systemic lupus erythematosus (SLE), multiple sclerosis and cancer[2]. Although the rate of progress in understanding the genetic basis of such diseases is now accelerating[3], the overall progress has been laborious, in part because of the clinical and genetic heterogeneity that is present in human populations, and in part because of the underlying intrinsic genetic complexity of the diseases. Moreover, even when the same genes are involved, the strength of their effects and their contribution to the overall phenotype can vary from individual to individual. The complexity of the immune system is mirrored, not surprisingly, in the multiplicity of controlling genetic elements and genetic interactions in these diseases.

Using mouse models of human diseases that are under complex genetic control alleviates the problems of genetic analysis. Genetic heterogeneity, for example, is reduced through the use of INBRED STRAINS, and the use of standardized housing conditions and diet can minimize environmental variation. Mouse geneticists have also built up a formidable armoury of experimental tools for teasing apart complex functional genetic interactions. In particular, consomic and congenic mouse strains are proving to be powerful tools for the study of immune disorders, and they are the main topic of this review. We explain the basis of these techniques and discuss how they contribute to our understanding of immune regulation and the aetiology of complex immune disorders.

Mouse models of human immune disorders

The present list of mouse disease models includes strains that carry single gene mutations — which can be of spontaneous origin, induced by radiation or, more often, the result of chemical mutagenesis[4,5] — as well as

RNA INTERFERENCE (RNAi). A technique in which the expression of a gene is inhibited when a double-stranded complementary RNA is introduced into the organism.

targeted knockout and transgenic mice (BOX 1). Another source of disease models is existing laboratory mouse strains[6,7] and it is these that, in many cases, provide excellent models for human diseases that are under complex genetic control. The Mouse Phenome Database (BOX 1) aims to add to the already remarkable range of phenotypes that are available by detailed, standardized and multifaceted phenotyping of some 50 mouse inbred strains through a coordinated international effort. This information should enable investigators to better identify appropriate strains for physiological testing, drug discovery, toxicology studies, mutagenesis, and investigation of disease onset and susceptibility, as well as new models for many human-disease-associated characteristics.

There has been considerable discussion about the relative advantages of using, as a means of identifying functional parts of the genome, phenotype-driven chemical-mutagenesis programmes that generate single-gene mutations affecting physiological processes, compared with using existing mouse strains that are models for changes in such processes[8]. In choosing an approach, it should be borne in mind that the chemical-mutagenesis screens are, by their nature, biased towards not only single-gene-based phenotypes, but also highly penetrant phenotypes. This might not matter if we are concerned mainly with 'the naming of biological parts', but it is surely of importance in terms of the models we use and in terms of understanding the integrative biology of mice. Resistance to the most widespread human diseases, such as cancer, autoimmune disorders and mental illness, is almost invariably modulated by a large number of genetic variants. Each of these variants confers subtle, poorly penetrant changes, which interact with other genetic and non-genetic factors in complex ways to produce substantive phenotypic variation. And nowhere is this more true than in the immune system with all of its molecular checks and balances, which are responsible, on the one hand, for mounting an effective immunogenic response to pathogens and, on the other hand, for maintaining a tolerogenic response to self-antigens. Moreover, it is increasingly clear that whole-animal chemical-mutagenesis programmes will, for various reasons, produce observable phenotypes in only a small percentage of the ~30,000 genes in the mouse genome[9,10]. Even in the nematode *Caenorhabditis elegans*, for which a systematic functional analysis of its genes has been undertaken by RNA INTERFERENCE (RNAi), fewer than 20% of the genes could be associated with a detectable phenotype[11,12]. And in a complex mammal such as the mouse, it is probable that an even smaller proportion of genes will give rise to detectable phenotypes. Constraints on the mouse strains that can be successfully mutagenized, for example, will clearly restrict the mutated genes that can be identified phenotypically. This is illustrated by the case of malaria-susceptibility genes, which have been studied recently by Fortin and colleagues[13] using crosses between A/J and C57BL/6 mouse strains. Although two C57BL/6 'resistance' genes could be detected in such classical crosses, the third C57BL/6 resistance gene on chromosome 3 could only be identified using a strain in which the first two C57BL/6 resistance genes had been replaced by alleles conferring sensitivity[13]. Therefore, although monogenic mutational models can be analysed with greater ease, a strong case can be made for the importance and relevance of studies that analyse mouse models of immune diseases that are under complex genetic control. Recent advances in our knowledge of the sequence and structure of the mouse genome (see later) are likely to be of particular value for the study of such models.

An important caveat to using mouse or other animal models to study diseases that are under complex genetic control is that analysis of identical human and mouse diseases might indicate that different genes are the main controlling elements. However, in such cases, the underlying molecular and cellular networks will still probably be common to all mammals. Therefore, an understanding of networks and pathways in mice will facilitate the design of targeted candidate-gene studies in humans, which can then be carried out on smaller, rigorously defined target populations[14].

Box 1 | **Helpful links for the use of mouse strains as disease models**

Human diseases
- http://www.ncbi.nlm.nih.gov/omim/

Mouse disease models
- http://jaxmice.jax.org/models/index.html

References for mouse models
- http://www.informatics.jax.org/external/festing/mouse/REFS.shtml/

Mouse models obtained by chemical mutagenesis
- http://www.emma.rm.cnr.it/
- http://www.gsf.de/ieg/groups/enu-mouse.html

Mouse phenome database
- http://www.jax.org/phenome/

Insulin-dependent diabetes mellitus: mouse models and congenic mice
- http://www.jax.org/t1dr/
- http://www.informatics.jax.org/

Single-nucleotide polymorphisms
- http://www-genome.wi.mit.edu/snp/mouse/
- http://mouseSNP.roche.com/

Bacterial artificial chromosomes
- http://www.bcgsc.bc.ca/projects/mouse_mapping/

Mouse sequence databases
- http://www.ncbi.nlm.nih.gov/
- http://www.informatics.jax.org/
- http://www.ensembl.org/
- http://mrcseq.har.mrc.ac.uk/
- http://www.genome.ucsc.edu/

Gene-expression data
- http://www.informatics.jax.org
- http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Mm

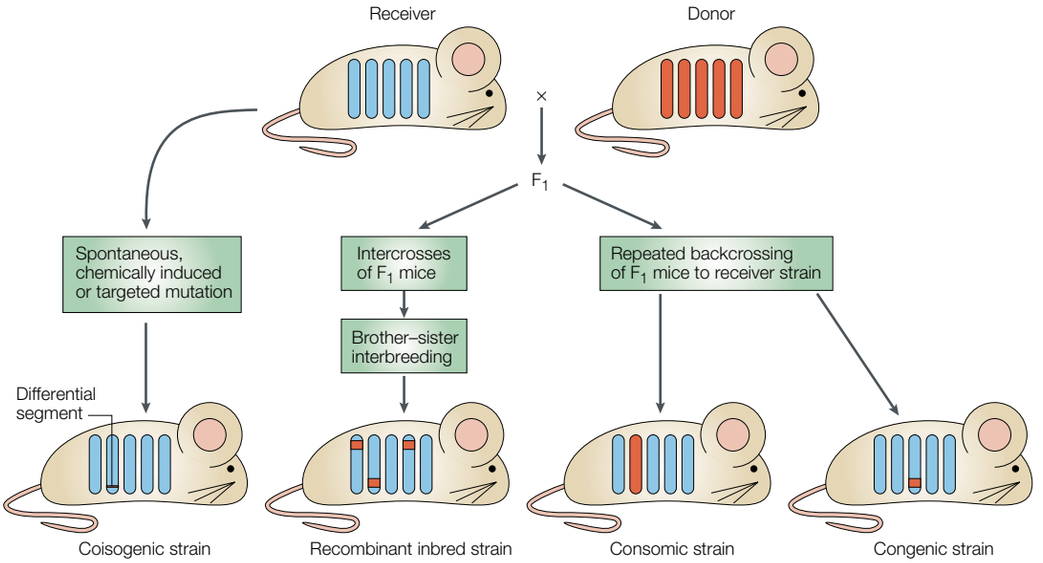

Figure 1 | **Selected analytical tools in mouse genetics.** The generation of coisogenic, recombinant inbred, consomic and congenic strains is illustrated.

What are congenic mice?

Congenic and consomic mice are special types of inbred strain in which part of the genome of one mouse strain is transferred to another, most often by backcrossing the donor mouse strain to the receiver strain with appropriate selection. In the case of a consomic (chromosome-substitution) strain, a whole chromosome is transferred[15,16], whereas in the case of a congenic strain, a defined chromosomal segment, the differential segment, is transferred[17,18] (FIG. 1).

In all cases, it should be appreciated that congenic strains will contain not only the selected differential locus, but also an associated length of the surrounding donor chromosome. Unless extensive breeding and testing of the congenic mice is carried out, the segment that is transferred together with the differential locus will often be at least several centimorgans (cM) in length and will contain several hundred genes derived from the donor strain. Only coisogenic strains[19], which are generally obtained by direct mutagenesis of an original inbred strain, will differ by a single defined locus (FIG. 1).

Congenic strains are used in two main ways. First, to standardize genetic-background effects in the study of a mutation. The PENETRANCE, or degree to which a mutation is expressed, and even the precise phenotype that is associated with many mutations, can vary with the genetic background on which they are expressed. The use of congenic strains, similar to inbred strains, allows repeat phenotyping of genetically identical individuals. Second, the use of congenic mice enables segments of the genome to be extracted from one genetic environment and placed in another, to dissect out and define the effect of a particular allele or HAPLOTYPE carried by the region of the mouse strain under study. Consomic mice provide a sort of halfway house, in that a whole chromosome is isolated from its usual genetic background and placed in another.

PENETRANCE
The proportion of affected individuals among carriers of a particular genotype. If all individuals with a disease genotype show the disease phenotype, then the disease is said to be completely penetrant.

HAPLOTYPE
An alternative form of a group of genes, part of a chromosome or a gene complex. The term is applied to groups of genetic loci, whereas the term 'allele' refers to alternative forms of a single gene.

Congenic mice and complex disease

One of the best studied mouse models of a complex immune disease is the non-obese diabetic (NOD) mouse, which spontaneously develops diabetes[20,21] with remarkable similarities to human IDDM. Progressive infiltration of the islets of Langerhans of the pancreas by antigen-presenting cells and lymphocytes leads to the onset of diabetes. As in humans, many genes are implicated in development of the disease. The MHC on mouse chromosome 17 was the first insulin-dependent diabetes (*Idd*) locus to be identified[22], and at least 20 additional *Idd* loci have been identified since then, at least in a preliminary manner, in crosses between the NOD strain and various diabetes-resistant strains[23]. Some of the *Idd* loci have been associated directly with other immunological anomalies that occur in NOD mice. For example, it has been shown that the candidate region for resistance of thymocytes to induced apoptosis overlaps the *Idd5* (REF. 24) and *Idd6* (REF. 25) loci.

As the penetrance of such multi-factorial diseases is low, mouse *Idd* loci, similar to their human counterparts, have proven to be relatively refractory to fine mapping by classical linkage-recombination analysis[26,27]. The construction of congenic strains is a powerful alternative approach for the dissection of this polygenic disease. We use the example of NOD mice to illustrate the application of congenic strains to immunology.

Congenic strains have been established for a large number of susceptibility/resistance loci for diabetes, with, for example, C57BL/KsJ, C57BL/10 or B6.PL-Thy.1a mice, or the NOD-related strain NON (non-obese non-diabetic) being variably used as the recurrent or background parental strain[28–32] (BOX 1). In certain cases, this has allowed particular *Idd* loci to be correlated with component subphenotypes of diabetes in

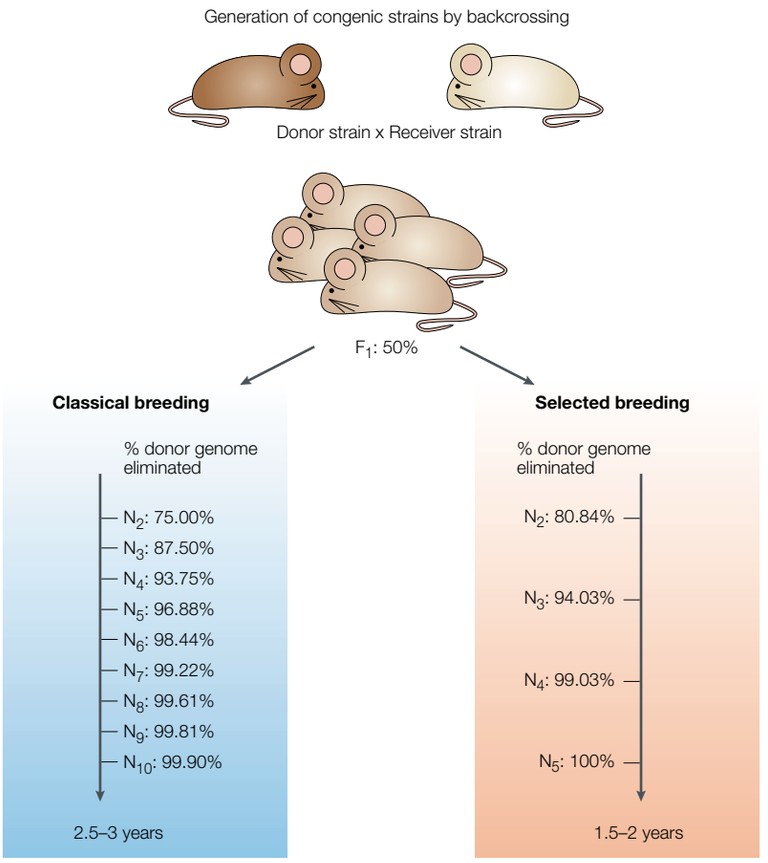

Figure 2 | **Comparison of classical-breeding and selected-breeding approaches to congenic-strain construction.** Statistical calculations indicate that, on average, classical-breeding approaches require nine backcrosses to eliminate fully (99.9%) donor-strain genome material outside of the differential segment from the receiver-strain genome that is selected for. Only four backcrosses are required when only the genetically 'best' animals from each generation (see text) are retained for backcrossing[37]. Normally, male animals are selected for, as they can produce larger numbers of progeny in the following generations than can females.

NOD mice. *Idd4* on mouse chromosome 11 has, for example, been shown to be involved in the control of T-cell proliferative unresponsiveness in NOD mice[33], whereas *Idd5* on chromosome 1 has been shown to control the progression of insulitis[34,35].

Non-NOD congenic strains include the GTM (genome-tagged mice) set, which contains more than 60 mouse strains, each carrying, on average, a 23-cM introgressed segment. In this congenic set, the C57BL/6J strain was used as the background strain with either DBA/2J or CAST/Ei mice used as the donor strain. The genetic basis of strain differences between donor and receiver can be mapped by simply characterizing all of the GTM strains for a given phenotypic trait and associating this with knowledge of the introgressed segment. Further fine mapping of the genetic determinant can be achieved by crossing the appropriate congenic mice to the background strain. Complex gene interactions can be investigated by studying combinations of the various congenic strains[36].

'Speed congenics'

Congenic strains are generally derived by repeated backcrossing of the desired donor strain to the recipient strain with selection for the differential segment, followed by sister–brother interbreeding of the backcrossed progeny (FIGS 1,2). When such repeated backcrossing is used to establish a congenic strain, a minimum of nine generations of backcrossing is normally recommended to remove unlinked and unwanted donor material, although the exact number of backcross generations is somewhat arbitrary[6].

New schemes involving both positive selection for the desired differential segment and selection against the rest of the donor genome amongst progeny of the early backcross generations allow congenic strains to be established much more rapidly. In essence, in such breeding schemes (known as 'speed congenics') the fact that heterozygosity amongst individual progeny of the N_2 (first backcrossed) and subsequent generations follows a normal distribution is exploited to select only the genetically 'best' animals — that is, those having the differential segment but minimal detectable donor-strain material elsewhere in the genome. Theoretically, this process can lead to the creation of a congenic strain with less than 0.5% contaminating donor genome unlinked to the differential segment in a total of five generations or four backcrosses[37] (FIG. 2). Thereby, the time required to establish a congenic line is effectively halved. Simulations indicate that screening between 16 and 20 male progeny per generation, with markers spaced every 25 cM, efficiently reduces the percentage of unlinked contaminating donor genome and is an effective organisational strategy. The use of larger progeny cohorts and higher marker density was of little advantage in reducing the percentage of contaminating donor genome until later backcross generations. High-density genotyping of the differential segment in later generations is, however, necessary to reduce the size of the target region below the 20–30 cM that is otherwise obtained by the N_4 generation[18]. As 'best males' do not always breed well, experience indicates that both 'best' and 'second best' males should routinely be kept for breeding. This is of particular concern for the construction of congenic strains between subspecies, when poor breeding performance might well occur. Such problems are encountered less frequently with classical congenic breeding, when both multiple matings and fortuitous selection for high viability might well occur.

Speed congenics has become not only an efficient method for generating standard congenic strains[18], but also an efficient approach for transferring targeted gene mutations rapidly to a disease-relevant background, as shown by the examples of ICA69 (null) NOD mice[38] and immunoglobulin-μ-null NOD mice[39].

The genetic interval that is covered by a particular congenic strain can often be reduced and refined by further backcrossing. However, there are certain obvious limitations to such interval reduction by direct breeding — the increasing difficulty in obtaining the necessary recombinants, the larger breeding populations that are required as the genetic distance is reduced, and the

admittedly more minor difficulty of finding informative polymorphic markers to identify such recombinants as the genetic distance is decreased.

Wild mouse strains and genetic diversity

Exploiting the genetic diversity of wild mouse strains through the use of inter-specific and inter-subspecies crosses is a superficially attractive way of increasing the number of informative polymorphisms in a cross. The use of wild mouse strains also allows a wider range of genetic variations that affect the immune system to be explored than does the use of standard laboratory inbred strains alone, and wild strains are an important source of new disease-resistance alleles[40,41]. However, marked divergence between strains creates problems of added genetic complexity for interpretation of the genetic analysis of traits that are under complex genetic control.

The advantages and disadvantages of establishing crosses using wild mouse strains can be better understood in the light of recent results concerning the distribution of genetic variation across the mouse genomes of inbred strains[42]. The genomes of many standard inbred strains seem to be a mosaic of megabase-sized regions, most of which are derived from either *Mus musculus domesticus* or *Mus musculus musculus*, which are the main ancestral population(s) of inbred strains. Regions of high genetic variation are interspersed with similarly large-sized regions showing little or no genetic variation, the latter corresponding to regions of recent common origin. The corollary of this is that whole tracts of genetic variation that are not present in the canonical inbred strains will be introduced when crosses involving inbred wild strains derived not only from *Mus spretus*, but also from *Mus m. domesticus* and *Mus m. musculus* are established.

The use of congenic strains derived from wild mouse strains has one other potential disadvantage. The high degree of polymorphism potentially complicates the later stages of the analysis, when it becomes a question of distinguishing causal from non-causal polymorphisms. For example, in non-coding regions, genetic polymorphism between laboratory strains, such as C57BL/6 mice, and inbred strains of the wild mouse *Mus castaneus*, which corresponds on average to one nucleotide difference per 80 nucleotides, is approximately tenfold greater than that between laboratory strains. This makes it difficult to separate the functionally important nucleotide differences from those without functional relevance. Also, inter-specific, and even inter-subspecific, mouse crosses often produce some sterile or semi-sterile offspring, particularly in the early generations of backcrossing, which makes the derivation of congenic strains difficult.

One QTL — several genes?

Dissecting a congenic segment that corresponds to a QUANTITATIVE TRAIT LOCUS (QTL) into subregions using congenic strains that cover only part of the original differential segment has provided surprising results on many occasions. An ostensible single locus might, in fact, contain several loci. Several examples of this have been noted for *Idd* loci: *Idd3*, *Idd10*, *Idd17* and *Idd18* on mouse chromosome 3 (REFS 43–45); *Idd9.1*, *Idd9.2* and *Idd9.3* on chromosome 4 (REF. 46); and *Idd6*, *Idd19* and *Idd20* on chromosome 6 (REF. 47). A corollary of this is that often the original phenotype that is being investigated is in fact a complex phenotype, which is at risk of being modified and becoming unapparent as the congenic interval(s) is refined. Although such linkage might be due, in part, to the ancestry of NOD mice and other mouse strains[42], evolutionary selection for fitness cannot be excluded. Tight linkage of QTLs, for example, does not seem to be restricted solely to mammals. Recently, a highly complex QTL architecture was discovered in yeast. The high-temperature growth (Htg) phenotype of clinically derived isolates of *Saccharomyces cerevisiae* has been found to depend on functional linkage both *in cis* and *in trans* of three tightly linked QTLs, which were shown to be neither necessary nor sufficient for this phenotype in isolation[48].

Other observations indicate that susceptibility genes involved in different autoimmune diseases might not be distributed randomly throughout the genome, but are clustered in certain genomic regions. For example, several susceptibility loci for diabetes and SLE are overlapping in NOD mice[49]. This non-random clustering supports the 'common-gene' hypothesis, which proposes that, in some cases, clinically distinct autoimmune diseases might be controlled by a common set of susceptibility genes[2].

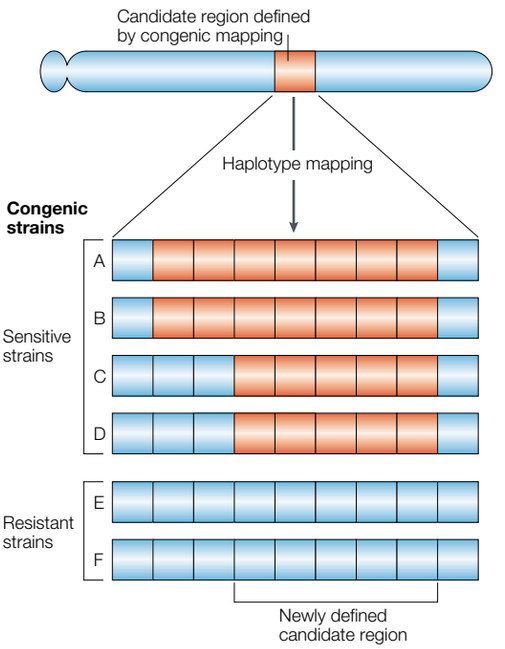

Figure 3 | **Haplotype mapping can help to reduce the size of a congenic candidate region.** The given candidate region can be further refined using haplotype mapping, which distinguishes between the distinct haplotype that is shared by disease-resistant strains on the one hand, and that of the disease-sensitive strains on the other hand.

QUANTITATIVE TRAIT LOCI (QTLs). Loci segregating alleles that have substantial input to the overall phenotype of a trait that is under complex genetic control.

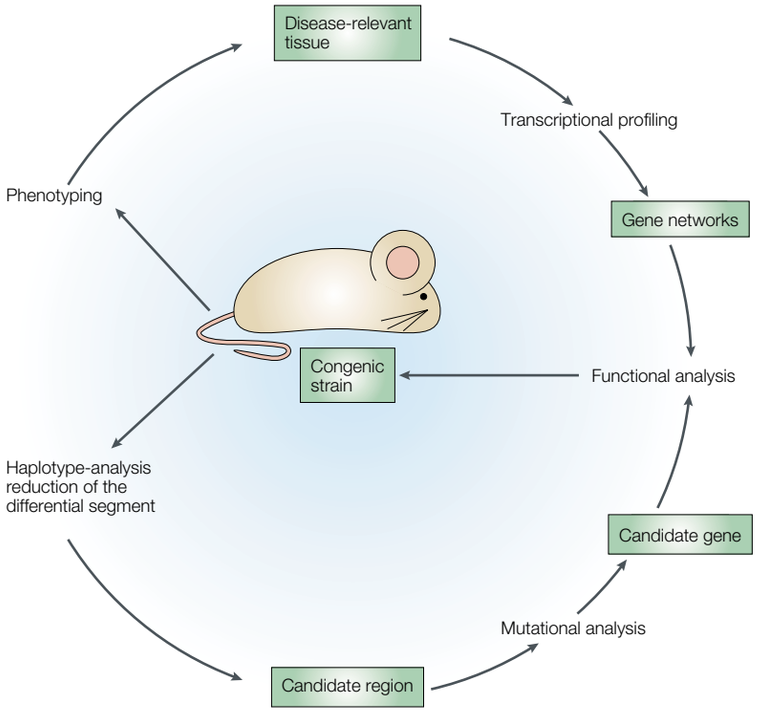

Figure 4 | **The pivotal position occupied by congenic strains in the analysis of complex traits.** Extensive phenotyping allows the definition of the disease-relevant tissue, which can then be used for transcriptional profiling. The candidate region defined by the differential fragment can be further refined using haplotype analysis. Genes identified by mutational analysis or by transcriptional profiling are subjected to functional analysis using the congenic strain.

Recombinant inbred strains as an alternative

Recombinant inbred (RI) strains are an alternative to congenic strains for the partitioning of individual complex traits into QTLs with sufficient strength to allow them to be studied as Mendelian loci (FIG. 1). In such strains, which are obtained by brother–sister breeding of selected F_2 mice, the genetic intervals derived from one or the other parent are fixed at random in the homozygous state. Although each RI line is an inbred strain in its own right, RI strains should be used as a group, as it is the pattern of characteristics displayed by the individual strains of the group — the strain distribution pattern — that is the most useful property of RI strains[6]. Although such RI strains have proven occasionally to be useful tools for the analysis of complex traits[31,50], they are generally better suited to the mapping of Mendelian traits[16]. The plans of the international mouse genetics community to generate advanced second-generation multi-parental RI panels will provide a valuable additional resource for the study of complex traits, as will recombinant inbred intercross (RIX) mice[51]. RIX mice are F_1 hybrids generated by crossing pairs of existing RI strains, which potentially offer greater genetic resolution than the parental RI lines. Similar to RI lines, RIX mice allow repeat phenotyping of genetically identical individuals, and this is aided by the tendency of RIX mice to facilitate more-reproducible phenotyping (due to lower error coefficients) than RI lines[51].

From congenic interval to candidate gene

The candidate region defined by a congenic strain can sometimes be further refined by assessment of the haplotype structure of the region concerned[52,53]. Recent work on the distribution of single-nucleotide polymorphisms (SNPs) in different mouse strains[54] (BOX 1) has shown that there is an alternating mosaic pattern of relatively large (typically 1–2 Mb) genomic regions (blocks) containing many or few SNPs[42]. Knowledge of the block structure of a congenic interval can indicate subregions of the congenic candidate region that are of particular interest. Genome-wide association studies in a wide range of inbred mouse strains involving the correlation of a phenotype — for example, disease susceptibility or disease resistance — to this genetic variation might provide additional information about potential candidate regions[55,56] (FIG. 3). This approach requires that parental-phenotype data be available for many inbred strains and that no mutation has arisen in a shared haplotype that could potentially lead to exclusion of the causative variant. Present plans to type SNPs systematically in many inbred mouse strains, including most of those that contribute to the Mouse Phenome Database, will be of importance in this respect.

Once the decision has been made to investigate the candidate region in detail, it is generally advantageous to use several complementary approaches in parallel (FIG. 4). The most direct approach is to use the mouse genomic sequence to assess the gene content of the region and then to resequence the identified genes or the entire region in the congenic strain to identify genes that show potentially important nucleotide variation. The increasingly well annotated mouse sequence databases (BOX 1) facilitate the exhaustive listing of genes in the candidate region. Where gene by gene resequencing is to be carried out, it is helpful to consider in detail the phenotype of the disease under study for clues as to the class(es) of genes or the expression profiles of the genes that might be involved. *In silico* expression profiles of the genes can be built up by identifying complementary DNA libraries that provide expressed sequence tags (ESTs) corresponding to the gene (BOX 1). Direct expression profiling and transcript quantification using technologies such as quantitative real-time PCR can also be carried out.

A particularly useful approach to identify genes and pathways that are involved in the disease mechanism is comparative expression profiling of genes in the candidate region between congenic strains. Global expression profiling using microarrays allows genes showing differential expression to be identified, which are then mapped back to the genome to identify those that localize to the differential congenic segment[57]. This strategy seems to be most promising when tissue complexity is reduced by analysing cellular subpopulations of the tissue[58,59]. Sensitive quantification of gene expression in complex tissues seems to require the use of relatively pure cell populations obtained by cell selection[60,61] or by laser-capture microdissection[62,63].

YEAST ARTIFICIAL CHROMOSOME (YAC). A large genomic fragment of up to 1 Mb in size, containing a centromere, an origin of replication and telomere sequences, that can be cloned into autonomously replicating yeast vectors. The genomic DNA fragments are maintained and propagated in the yeast *Saccharomyces cerevisiae* as linear chromosomes.

BACTERIAL ARTIFICIAL CHROMOSOME (BAC). A cloning vector derived from a single-copy F-plasmid of *Escherichia coli* that carries the F replication and partitioning systems that ensure low copy number and faithful segregation of plasmid DNA to daughter cells. Large genomic fragments can be cloned into such vectors and they are faithfully replicated, which makes BACs useful for constructing genomic libraries.

Alternatively, functional tests — for example, the use of YEAST ARTIFICIAL CHROMOSOMES (YACs) and/or BACTERIAL ARTIFICIAL CHROMOSOMES (BACs) to transfer the trait under analysis — can be used to reduce the size of the candidate region[56,64]. Given the size of the candidate region, a useful organizational compromise is to use pools of BACs or YACs, which are transferred onto a suitable genetic background. Such approaches are being facilitated by the construction of BAC libraries for mouse strains other than C57BL/6 and 129/Sv (BOX 1) and by the ready availability of the mouse draft genome sequence[65] (see later), which allows the DNA hybridization probes that are necessary for BAC isolation to be easily designed. Although this trangenic approach is efficient for selecting genomic clones containing disease-relevant genes, it should be noted that the integration of YACS and BACs in the genome can occasionally lead to modifications in gene expression owing to position effects, and that this can then hamper the identification of genes controlling subtle phenotypes.

Functional tests for genes

Technologies that seem to be suitable for the verification of strong candidate genes include transgenesis, gene knockout and deletions of larger chromosomal regions generated by genetic engineering[66]. Transgenics have been created for single genes, such as the glutamate decarboxylase (*GAD*) gene, which encodes an antigen that is important in IDDM[67], but they are probably most usefully constructed using larger genomic clones, such as BACs, which allow the immediate genomic environment to be more faithfully established in the transgenic mouse lines[68]. Efficient methods for modifying such BAC clones for the transfection of mammalian cells are now available[69]. Knockout mice are mostly generated on the 129/Sv inbred strain background, although embryonic stem (ES)-cell lines of the C57BL/6 strain, which is more commonly used by immunologists, are now available[70]. ES-cell lines are, however, unfortunately not available for all mouse inbred strains, including many that are important in immunological research. Little progress, for example, has been made in establishing ES-cell lines from NOD mice[71], although recently, ES-cell lines have been established from NOD/129Sv F_1 mice[72]. As, in many cases, the 129/Sv knockouts are not directly suitable for the analysis of immunological diseases, backcrossing onto the relevant genetic background is necessary[72]. Differences in trait penetrance are, as noted earlier, frequently observed during such backcrossing, and tightly linked genes might not be separated easily.

New technologies, such as RNAi, that block gene translation and/or transcription[73–79] should facilitate more refined functional genetic analysis and might replace, at least in part, knockout technologies now that systems for the conditional expression of the inhibitory RNA are becoming available. RNAi seems to be particularly promising as a solution for the problems that are posed by genetic background, as it should work on most, if not all, genetic backgrounds.

Congenic mice and other immune QTLs

Congenic mice have not only helped in the understanding of IDDM, but have greatly advanced our knowledge of other important autoimmune diseases, such as SLE. This disease, which is characterized by the production of immunoglobulin G specific for nuclear constituents (which seems to lead to damage to many organs), shows extreme diversity between individuals in terms of its clinical manifestations and the levels of associated autoantibody production[80]. New Zealand black (NZB) and New Zealand white (NZW) mice are well-studied models of lupus nephritis and of pathogenic DNA-specific IgG autoantibody production. At least twelve non-MHC loci have been associated with the aetiology of SLE in mice. A locus on mouse chromosome 1, known as *Sle1*, has been shown to have remarkable linkage to the disease, and it is the main susceptibility locus in the New Zealand mixed (NZM)-derived RI strain NZM2410. When isolated on a C57BL/6 background in the C57BL/6.*Sle1* congenic strain, *Sle1* results in the production of high levels of chromatin-specific IgG antibodies, histone-specific T cells, and increased B- and T-cell activation[81]. A second locus, *Sle2*, lowers the activation threshold of B cells, whereas a third locus, *Sle3*, mediates the dysregulation of $CD4^+$ T cells[82,83]. A more detailed genetic analysis of the *Sle1* locus on mouse chromosome 1 using the C57BL/6.*Sle1* congenic strain[84] has shown that it is in fact composed of at least three loci, known as *Sle1a*, *Sle1b* and *Sle1c*, lying in the same congenic interval, which can independently cause a loss of tolerance to chromatin.

Recently, studies in mouse experimental models have also contributed greatly to our understanding of the mechanisms of allergic inflammation that underlie asthma[85,86]. Bronchial asthma is one of the most common chronic diseases affecting children and young adults. A crucial phenotypic characteristic of human asthma and an important feature of animal models of asthma, such as A/J mice, is airway hyperresponsiveness[86]. Mouse models have shown the role of IgE in the inflammation process[87], and the importance of T helper 2 (T_H2) cytokines[88] and of interleukin-5 (IL-5)[89]. T_H2-type cytokines are encoded by genes found on human chromosome 5q23–q35, which is homologous to a region on mouse chromosome 11. McIntire *et al.*[90] recently examined a congenic strain known as HBA, which contains a segment of mouse chromosome 11 inherited from DBA/2 mice — which have low-level T_H2 responses — transferred onto the high-responder BALB/c background. They identified a Mendelian trait implicated in the development of airway hyper-reactivity and T-cell production of IL-4 and IL-13 that is controlled by the T-cell and airway phenotype regulator (*Tapr*) locus, which is genetically distinct from all known cytokine genes[56]. Positional cloning identified a gene family encoding T-cell membrane proteins (TIMs). Important sequence variants of this gene family (*Tim*) were shown to be co-segregated with *Tapr*. *Tim1* polymorphisms correlated with the development of higher-level T_H2 responses in BALB/c mice than in HBA mice. A human homologue of *Tim1* (*TIM1*) has

EPISTASIS
When the phenotype caused by a mutation in one gene is masked or enhanced by a mutation in another gene.

been identified and shown to be the hepatitis A virus (HAV) receptor. The authors[90] propose that the interaction of HAV with human TIM1 might reduce T_H2-cell differentiation and the probability of developing asthma.

A large number of mouse models for other immune disorders exist already, including the K/BxN T-cell receptor-transgenic mouse, which is a model of inflammatory arthritis similar to rheumatoid arthritis[91], and the MRL/lpr mouse, which spontaneously develops various forms of autoimmune disease in the same individual, including glomerulonephritis, polyarteritis, arthritis and sialoadenitis[92]. Much ongoing research is aimed at defining the genetics of autoimmune aetiology in such models.

Immune networks in humans and mice

Understanding the genetic factors that are involved in autoimmune or allergic diseases is clearly no easy matter. Disease-relevant polymorphisms and mutations might remain undetected in humans owing to the large genetic variation that is present in the population, and in both humans and animal models, genetic interactions between genes can mask individual genetic effects[93].

One of the main challenges in the coming years will be to characterize and understand the genetic interactions that contribute to immune disease. Such analysis will depend on the parallel use of transcriptome and proteome analyses, and on systematic functional immunological characterization in combination with genetic systems allowing the deletion, modification and switching between mouse strains of different components of the system. To analyse efficiently the effects of such gene interactions and EPISTASIS using congenic strains, it will clearly be an advantage to use congenic mice carrying the smallest possible genetic interval, as this will substantially reduce the number of genes that have to be considered. However, because of the limitations that are involved in deriving congenic strains containing genetic regions of less than 2 Mb in size, which corresponds to ~1 cM, it is probable that the use of congenic mice for such studies will be assisted by homologous recombination-based techniques that allow 'allele shuffling', by highly efficient systems of gene mutation and, increasingly promisingly, by techniques, such as RNAi, that allow allele-specific inhibition of gene expression[94]. As our knowledge of the functional organization and regulation of the genome improves and as we begin to understand the workings of the transcriptome[95] and proteome, such interactions in the immune system might, for the first time, become fully interpretable in molecular terms. As genetic components of the immune system, such as cytokine genes, are amongst the most rapidly evolving of all known gene families, the evolutionary stability of such networks between different mammals will be of particular interest. The efficiency and overall stability of our immune system indicates that the redundancy in its organization is probably paralleled by a type of evolutionary opportunism in the grafting on and use of unrelated genetic components with originally diverse functions that has been referred to as evolutionary 'tinkering'.

Concluding remarks

Congenic strains have a pivotal role in efforts to understand the genetic complexity of the immune system and in the study of its dysfunction in autoimmune diseases using defined mouse models. Examples of such diseases that we have discussed in some detail include IDDM, arthritis, SLE and airway hypersensitivity[29,91,92,96]. Congenic strains are also likely to have an important role in at least the initial phases of studies into the genetic interactions that underlie immune function, through the intercrossing of different congenic mice or of congenic mice with immune-gene knockouts, as well as transgenic mice[97], coisogenic strains and strains carrying particular mutations. A more complete understanding of the complexity of the genetic and epigenetic networks underlying the immune system in mice will, in turn, facilitate modelling of the human immune system and immune disorders in humans, and an appreciation of the differences between humans and mice with regard to the functioning of the immunological response.

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