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Mouse models as a tool to unravel the genetic basis for human otitis media

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Abstract

The pathogenesis of otitis media (OM) is multifactorial and includes infection, anatomical factors, immunologic status, genetic predisposition, and environmental factors. OM remains the most common cause of hearing impairment in childhood. Genetic predisposition is increasingly recognized as an important factor. The completion of the mouse genome sequence has offered a powerful basket of tools for investigating gene function and can expect to generate a rich resource of mouse mutants for the elucidation of genetic factors underlying OM. We review the literature and discuss recent progresses in developing mouse models and using mouse models to uncover the genetic basis for human OM.

Keywords

Otitis media; Mice; Genetic predisposition to disease; Etiology

1. Genetic factors underlying otitis media in humans

Otitis media (OM) is a multifactorial disease whose pathogenesis is affected by Eustachian tube (ET) structure and function, immune status, innate mucosal defense, pathogens, and, importantly, genetic susceptibility loci. Infectious disease can be viewed as a battle between hosts and pathogens, in which commands encoded in the genomes of both host and pathogen are executed by protein products that include components of the host immune response and drug resistance mechanisms of the bacterial pathogen. Although genetics may not be considered as a factor in the development of an infectious disease such as OM, many lines of evidence indicate that the genetic background of the host plays an important role in OM. For example, patients with recurrent OM usually exhibit some of the following characteristics: sibling history of frequent ear infections, Down syndrome, cleft palate, and immunodeficiency (Daly et al., 1991, 1999a,b). Racial differences also suggest a genetic contribution to OM susceptibility. OM frequency is unusually high in American Indians and Australian Aborigines and comparatively low in African Americans (Coates et al., 2002;Harris et al., 1998). A study of OM in Apache Indians in Arizona also suggests familial predisposition (Todd et al., 1987). Some of the most compelling evidence comes from a twin and triplet study which concluded that genetic traits play a major role in OM development and that OM susceptibility is inherited (Casselbrant et al., 1999). Various congenital and inherited syndromes also demonstrate a genetic influence on OM susceptibility. For example, one study found that 89% of 193 children with achondroplasia had at least one episode of OM within the first 2 years of life and that 24

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of the 99 children who had OM in the first year of life had multiple episodes (Hunter et al., 1998).

Studies of several human syndromes have also contributed to identifying genes that might be involved in predisposition to OM. Kartagener's syndrome is an autosomal recessive heritable disorder with impaired function of the mucociliary system of the Eustachian tube. In a study of Kartagener's syndrome, all 27 affected children developed chronic sinusitis and OM (Mygind et al., 1983). More recently, mutations in the dynein heavy-chain gene (DNAH5) were identified in Kartagener's syndrome families, aided by genetic mapping information of the homologous gene in the mouse Mdnah5 (Olbrich et al., 2002; Vaughan et al., 1996). Indeed, a null mutation of the Mdnah5 mouse gene exhibited the OM phenotype. Gene expression studies have demonstrated that surfactant protein A, which plays a role in innate host defense in the lung, is also expressed in the Eustachian tube (Ramet et al., 2001).The frequency of specific surfactant protein A haplotypes and genotypes has been shown to differ between children with recurrent OM and those in a control population in Finland (Alho et al., 1991).

Although the above studies suggest that genetic factors contribute to OM, human genetics approaches are limited in the ability to undertake systematic investigations of the genetic pathways and pathological mechanisms involved in middle ear disease. For example, genome-wide association studies in human populations with the aim of identifying genetic loci underlying OM are fraught with significant logistical and practical difficulties. Moreover, genetic investigations in the human population are compounded by uncontrollable environmental factors. While none of these difficulties is completely insurmountable, there are significant advantages to the parallel development of mouse models of OM. The mouse can play a key role in unraveling the genetic etiology of OM, information that can be translated to studies of the genetics of OM in the human population — for example, by assessing candidate genes identified in the mouse in association studies in human families. Moreover, a diverse panel of mouse genetic models will provide an important platform for drug discovery and the development of alternative therapeutic strategies for human OM.

2. Pathogen challenge induced otitis media mouse models

Several animal models of OM have been reported, including the chinchilla (Bakaletz et al., 1998; Giebink et al., 1999) and the rat (Clark et al., 2000). Animal models, including mice, have been used successfully to elucidate virulence factors, mechanisms of bacterial adherence and invasion, induction of mediators of inflammation, and the specificity of immune responses to pathogens such as nontypeable Haemophilus influenzae (NTHi) (Green et al., 1993; Kyd et al., 1995; Wallace et al., 1989), H. influenzae type b (Loeb et al., 1987), Pseudomonas aeruginosa (Cripps et al., 1994), Streptococcus pneumoniae (Yamamoto et al., 1997), and Moraxella catarrhalis (Kyd et al., 1999). Increasingly, mice have been used to study OM because of the commercial availability of immunologic probes (Gu et al., 1996, 1998; Johnson et al., 1997; Klingman et al., 1994; Krekorian et al., 1990; Kyd et al., 1999; Murphy et al., 1999). Non-genetic mouse models of OM have been generated in approximately 100 studies, of which over 30% used the BALB/c inbred strain (Chen et al., 1996; Gu et al., 1995; Hotomi et al., 2002; Ichimiya et al., 1999; Kataoka et al., 1991; Klingman et al., 1994; Krekorian et al., [1990], [1991]; Kurono et al., 1992; Kyd et al., 1999; Murphy et al., 1999; Sarwar et al., 1992; Ward et al., 1976; Watanabe et al., 2001). Mice have also been inoculated with bacteria or their cell-wall antigens to induce immunities for studies of pathogen–host interactions (Gu et al., [1996], 1998; Holmes et al., 2001; Hotomi et al., 1998; Klingman et al., 1994; Kodama et al., 2000; Krekorian et al., 1990, 1991; Murphy et al., 1999; Sarwar et al., 1992). In studies using BALB/c mice, a detoxified lipopolysaccharide (LPS)–protein conjugate is a suggested candidate for immunization against M. catarrhalis infection (Gu et al., 1996, 1998). None of the studies with OM models mentioned above addressed the underlying genetic basis for host

susceptibility. However, the most significant advantage of using mouse OM models over other model organisms is that there is an extensive genetic toolkit available for manipulating the mouse genome and studying the relationship between genes and disease susceptibility (Cox et al., 2003; Parkinson et al., 2002; Whitfield et al., 2005). For example, a significant difference in middle ear inflammation and effusion formation in response to Gram-positive bacterial cell wall product (peptidoglycan-polysaccharide) was reported between two genetically different strains of rats (Clark et al., 2000). If this work had been carried out in the mouse, the gene (s) underlying the observed differences could have been more easily identified due to the well-developed high-resolution mapping, positional cloning, and genetic manipulation tools available.

3. Genetic approaches to studying mouse models of otitis media

The completion of the mouse genome sequence has enabled a more or less complete annotation of mouse genes (Waterston et al., 2002). This development coupled with the tools to introduce defined, targeted alterations into the mouse genome means that we are able for the first time to contemplate undertaking a systematic assessment of gene function using large-scale mouse mutagenesis, phenotype assessment of mutant strains, and the identification of disease models from the mutants created.

There are two distinct approaches to determining gene function in the mouse — gene-driven and phenotype-driven (Bradley et al., 1984; Gu et al., 1993; Hrabe de Angelis et al., 2000; Nolan et al., 2000; Thomas et al., 1987; Wiles et al., 2000). In the gene-driven approach, a specific lesion introduced into the mouse genome is the start point for an analysis of the resulting phenotype. Gene-driven approaches include genetraps and knock-out and knock-in mutations (Bradley et al., 1984; Gu et al., 1993; Thomas et al., 1987; Wiles et al., 2000). It is feasible for such targeted approaches to be scaled in order to generate mutations for every gene in the mouse genome. Moreover, targeting constructs can be manipulated in order to introduce conditional mutations so that mutational effect can be explored in both a time-dependent and tissue-specific manner (Gu et al., 1993). There has been much recent discussion on the development of international programs to generate mutant lines for all mouse genes (Austin et al., 2004; Auwerx et al., 2004). In Europe, the EUCOMM (European Conditional Mouse Mutagenesis program) will undertake the generation of conditional mutations for 20,000 genes and begins in 2006. In Canada, a similar program (NORCOMM) will also get underway shortly, while in the US, the KOMP (Knock-out Mouse program) initiative is considering proposals. The mutant lines produced from all of these programs will be a major resource for studying gene function and generating diverse disease models.

In contrast, the phenotype-driven approach undertakes screens of large collections of randomly mutagenized mouse genomes, commonly produced by chemical ENU mutagenesis, for disease phenotypes of interest (Hrabe de Angelis et al., 2000; Nolan et al., 2000). Thus, the phenotype of interest is the start point of the study irrespective of the underlying lesion responsible. Having discovered an interesting mutant phenotype, the underlying gene is identified and investigated further. Importantly, phenotype-driven approaches do not make any a priori assumptions about the relationship between gene and phenotype and are therefore a relatively powerful route for discovering novel gene function and genetic pathways. Thus, in the case of OM, where the genetic etiology is very poorly understood, this approach would be expected to yield benefits. An additional advantage of ENU is that it introduces point mutations and has the capacity to reveal many of the gene–phenotype relationships at an individual locus by the introduction of a range of null, hypomorphic, gain-of-function, and dominant-negative mutations.

Taken together, gene-driven and phenotype-driven approaches offer a powerful basket of tools for investigating gene function and can expect to generate a rich resource of mouse mutants

for the elucidation of genetic mechanisms underlying disease. Indeed, a phenotype-driven approach has recently been proven instrumental in the identification of pathogen susceptibility and resistance genes and their alleles. In each case, the work began by identifying mouse strains showing differing susceptibility to infection. In the first example, a 2′-5′-oligoadenylate synthetase 1B gene (Oas1b) was identified as Flv (flavivirus resistance gene) via analysis of the association between genotype and phenotype in nine mouse strains differing in susceptibility to flavivirus infection (Perelygin et al., 2002). This study showed that susceptible strains produce a protein lacking 30% of the C-terminal sequence due to a premature stop codon. In a second study, mouse strains susceptible and resistant to West Nile virus were identified and used to map the susceptibility locus. A C-terminal transition that results in a stop codon in exon 4 of a gene encoding the L1 isoform of Oasl (Oasl1) was identified as the cause of susceptibility in laboratory mice (Mashimo et al., 2002). These studies give confidence that the development of genetic mouse models of OM would contribute significantly to efforts to identify the genetic and biologic factors impacting OM. Moreover, having identified and characterized mouse models, we can investigate the epistatic effects of mutations by performing crosses between different mutant strains and thus further explore the multifactorial basis of disease (Zheng et al., 2001).

4. Current state of mouse models in research

BALB/c mice have been used extensively in OM research, both in pathogen challenge experiments and immunologic studies, primarily investigations of vaccine efficacy. Several molecules were identified in middle ear effusions as inflammatory mediators, including the cytokines IL-1β, IL-2, IL-6, IL-8, TNFα, interferon-γ, and TGFβ (Ball et al., 1997; Maxwell et al., 1997; Nassif et al., 1997; Storgaard et al., 1997). The results of these studies indicate that these mediators play an important role in the pathogenesis of OM. It is possible that genetic differences between mouse strains could affect the production of these mediators as part of the immune response to pathogens, which, in turn, could affect susceptibility to OM (see also below). Studies using Swiss–Webster, BALB/c, or CD-1 mice have shown that bacterial adherence factors, including NTHi, surface protein pneumococcal surface adhesin A (PsaA), ubiquitous surface protein (Usp)A1, and ubiquitous surface protein A (UspA)2, may have some role in adherence (Briles et al., 2000; Chen et al., 1996; Holmes et al., 2001; McMichael et al., 2000). Gene knock-out mouse models (http://www.jax.org/imr/index.html) have been produced in most genes of the molecules mentioned above, such as IL-1β, IL-2, IL-6, IL-8, PsaA, UspA1, and Uspa2, but few of the knock-out mouse models have been used for OM studies.

Other examples of mice used in OM research include studies of signal transduction mechanisms involved in OM pathogenesis (Bakaletz et al., 2002). For example, receptor tyrosine kinases were demonstrated to be involved in normal and pathologic angiogenesis in mice (Sudhoff et al., 2000). It was found that regulatory genes involved in middle ear development may also influence susceptibility to OM.

Several groups have used potential genetic mouse models to study OM. Inbred strains (C3H/HeJ (Mitchell et al., 1997), outbred Mcr:(ICR) breeder mice (Harkness et al., 1975), and B6;129 mice (Haines et al., 2001)) have been reported to have relatively high incidences of OM. Several CBA/J colonies had to be destroyed because the incidence of purulent OM was as high as 62% (McGinn et al., 1992), and CBA/CaJ was recommended as a replacement to provide appropriate controls in hearing studies. There have been more than a dozen middle ear studies using LP/J mice (Brodie et al., 1993; Henry et al., 1987; Steel et al., 1987), but none of these studies included genetic characterization of the OM susceptibility traits. Similarly, some studies have generated pathogen-challenged OM models in BALB/c mice, but none of these was designed

to identify the gene(s) affecting host susceptibility to OM (Chen et al., 1996; Kyd et al., 1999; Sabirov et al., 2001).

A possible reason for the current lack of a well-developed genetic mouse models of OM is that many existing mouse strains may be resistant to middle ear infections (Mitchell et al., 1997). However, to date, no systematic screening has been performed for OM, as has been done for other disease susceptibilities (Alper et al., 2002; Chang et al., 2002; Mashimo et al., 2002; Nolan et al., 2000; Perelygin et al., 2002; Zheng et al., 1999). In particular, no effective rapid screening methodology has been established for the detection of OM in the mouse. Nevertheless, screening of large numbers of mutant mice from ENU mutagenesis programs for hearing impairment using relatively simple but high-throughput procedures such as the click-box test might be expected to identify mutants showing a conductive hearing loss along with mice with sensorineural hearing defects (Brown et al., 2003). Indeed, the discovery of the deaf mouse mutant Jeff (Jf), a single locus model for OM, in an ENU program in the United Kingdom underlines the potential of large-scale mutagenesis projects for the identification of OM mouse models (Hardisty et al., 2003). The Jf mutant shows a significant conductive hearing loss by postnatal day 35 with fluid and pus in the middle ear cavity. Jf mice develop a chronic suppurative OM with severe inflammation of the mucoperiosteum. The Jeff locus maps to mouse chromosome 17. Another mutant, Junbo (Jbo), with a very similar phenotype has been identified from the same ENU mutagenesis program. Junbo maps to chromosome 3, and, recently, a mutation in the gene encoding the transcription factor Evi1 has been shown to underlie the OM phenotype (Hardisty-Hughes and Brown, unpublished data). This is a very provocative finding since the Evi1 transcription factor is a repressor of the TGFβ pathway which is already implicated in OM pathogenesis and from in vitro studies has been shown to be involved in signaling pathways controlling mucin production (see above and Hardisty et al., 2003). Both these studies emphasize the potential benefits of continuing to screen large-scale ENU programs to identify additional models of OM.

Studies employing gene targeting or other transgenic modifications also support the feasibility of developing additional valuable genetic mouse models of OM. Chronic OM is commonly found in VCFS/DGS patients with 22q11 deletions (Funke et al., 2001). Mice overexpressing genes from this region showed OM. Mice harboring a homologous deletion of the p73 locus had a 100% incidence of OM on a C57BL/6 background. A feature of $p73^{-/-}$ mice (Yang et al., 2000) was purulent OM at the earliest ages (post natal day 2 pups), which persisted through adulthood. Microbiologic analysis of affected sites from $p73^{-/-}$ weanlings (P21) revealed the presence of Escherichia coli, Pasteurella aerogenes, and micrococcal species. Despite these indications of inflammation and infection, no obvious deficiencies in lymphoid or granulocyte populations were detected in $p73^{-/-}$ mice, indicating that there might be defects in other components of the natural immune system. Mice deficient in lymphocyte function associated antigen 1, $LFA\text{-}1^{-/-}$ (CD11a/CD18), have increased incidence of OM but also have significantly increased mortality (Prince et al., 2001). In addition to mice with spontaneous and ENU-induced mutations, The Jackson Laboratory maintains more than 1000 existing transgenic and knock-out mouse strains, which are freely available for screening for genetic models of OM.

5. Conclusions

There can be much optimism that the genetic analysis of mouse models of OM will lead to the identification of novel loci in mouse and humans and a more profound understanding of the genetic etiology of this complex, multifactorial disease. The identification of these loci and the study of the specific genes involved might offer the possibility of screens to predict OM susceptibility, which will assist physicians in identifying those patients who are at risk for severe OM and, therefore, may benefit from prophylactic or targeted treatment. Moreover, the

development of a new tranche of mouse mutants and a better understanding of the genetic basis for OM will deliver opportunities to devise novel therapies as well as provide relevant models for pre-clinical testing.

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