Searching for Genes of Host Defense

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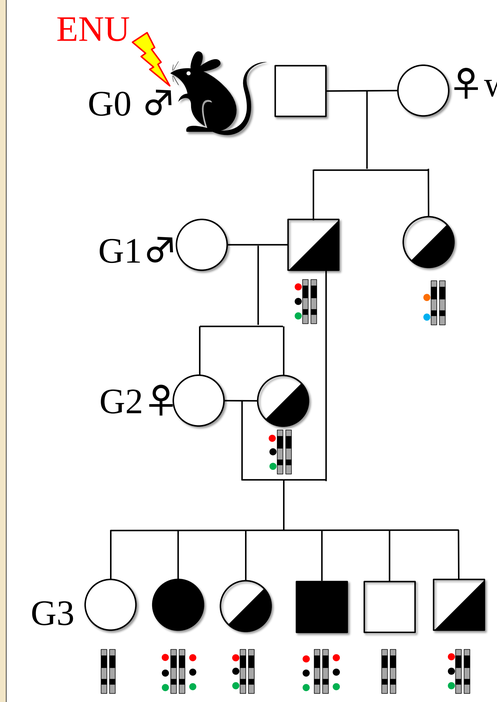
ABSTRACT

Through random mutation of the mouse genome and phenotypic screening of the mutated mice, genes can be identified that are associated with dysfunction in the innate immune system. The strategy proposed works under the knowledge that many genes are involved in the immune system and that random mutation could lead to a change in their genetic code. This mutation can present as a phenotypically abnormal immune system. Once a phenotype is identified, the genome can be analyzed in an attempt to trace the mutated gene responsible for the weakened immune system. One of the elegant aspects of this genetic method is that it does not rely on a hypothesis about how the immune response works. This leads to an unbiased approach where interpretation errors are rarely made.

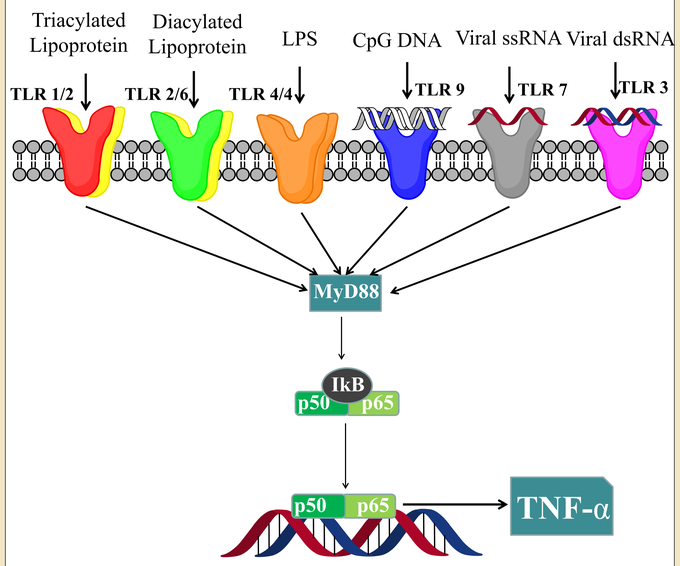
After genomic mutagenesis, mouse macrophages were screened for genetic defects in the Toll-Like Receptor and Inflammasome pathways.

INTRODUCTION



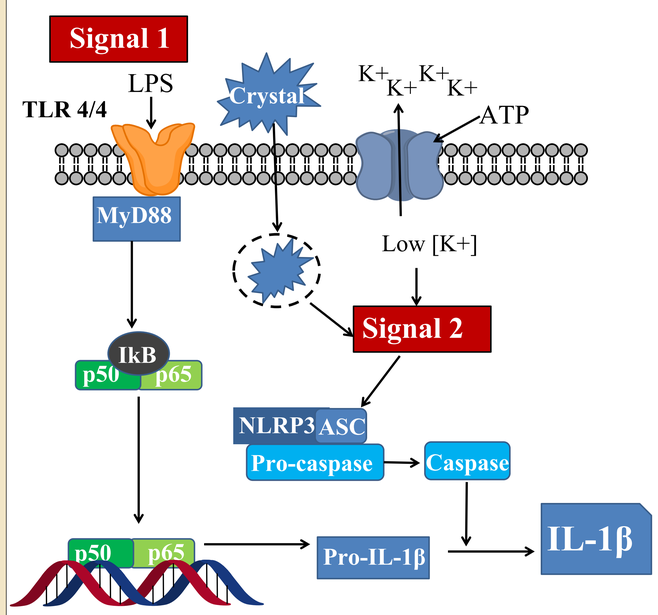
A forward genetic approach is used to create abnormal phenotypes of the innate immune system and then determine the genetic cause. The normal mutation rate is accelerated by the widely used germline mutagen N-ethyl-N-nitrosourea (ENU) to produce an average of 3,000 single nucleotide changes per host leading to an average of 60 coding changes. To produce homozygotes, males of the G1 generation are bred with normal mice of the same strain to yield the G2 generation. Recessive mutations can be found in the G3 generation by a backcross of G2 females with the G1 father. Screening 6 G3 progeny should capture 50% of the mutations in the homozygous form.

Toll-Like Receptor Screen



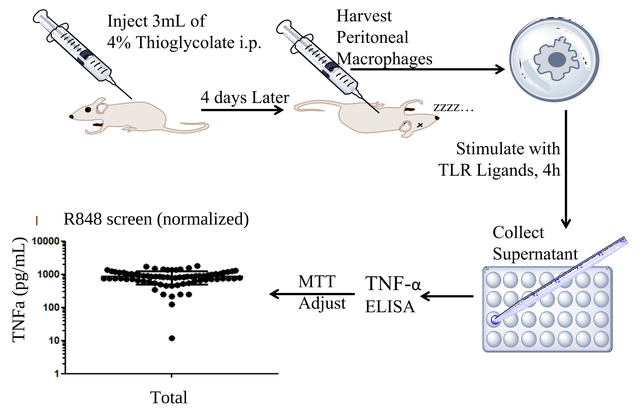
Phenotypic screening was performed on peritoneal macrophages ex vivo by stimulation with the following toll-like receptor agonists: Lipopolysaccharide (TLR4), Double stranded RNA (TLR3), Triacylated lipoprotein (TLR 1/2), Diacylated lipoprotein (TLR 2/6), Resiquimod (TLR 7), and Unmethylated DNA (TLR 9). Secreted TNF-alpha was measured to determine phenovariance.

Inflammasome Screen



Phenotypic screening was performed on peritoneal macrophages ex vivo by priming with Lipopolysaccharide (Signal 1), then stimulating with Nigericin (K+ efflux) or exogenous ATP. Secreted IL-1beta was measured to determine phenovariance

Screening Process



The TLR and inflammasome screening of peritoneal macrophages was performed on 668 G3 mice from 132 matings of G2 females with G1 males. The average litter size was 5 pups per mating and there was an average of 3 G2 females mated with G1 fathers. Screening of this set of G3 peritoneal macrophages yielded no significant decrease in TNFα or IL-1β secretion in response to the TLR and inflammasome screenings applied.

CONCLUSION

A forward genetic approach was used to create abnormal phenotypes of the innate immune system and then determine the genetic cause. Phenotypic screening was performed on peritoneal macrophages ex vivo by stimulation with the TLR agonists and stimulators of the inflammasome.

This research can lead to a deeper understanding of how we combat infection. The study can lead to the development of mutations involved in both the innate and adaptive immune system so autoimmune diseases can also be studied. A long term goal is to identify genes that would render an individual resistant to infection and to study the interaction of these genes.

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