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The inducible nitric oxide synthase gene, *Nos2*, maps to mouse Chromosome 11

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Synthesis of nitric oxide (NO) from L-arginine and molecular oxygen is catalyzed by nitric oxide synthase (NOS). Two constitutive isoforms (Bredt et al. 1991; Lamas et al. 1992) and one inducible isoform (Lyons et al. 1992) have been isolated and purified; it is unknown whether other isoforms exist. One human constitutive NOS gene, NOS1, is expressed in neurons and maps to 12q24.2-24.31 by in situ hybridization (Xu et al. 1993). The other human constitutive NOS gene, NOS3, is expressed in endothelial cells and maps to 7q35-36 (Marsden et al. 1993). The cytokine-inducible isoform has also been described in humans and rodents; the structural locus (NOS2) maps to human Chromosome (Chr) 17p11-17q11 (Charles et al. 1993; Geller et al. 1993a), but the mouse locus *Nos2* has not yet been mapped.

Inducible NOS has been found in a wide variety of cell types, including macrophages (Stuehr and Marletta 1985), endothelial cells (Gross et al. 1991), hepatocytes (Curran et al. 1989), and a pancreatic beta cell line (Eizirik et al. 1992). Expression of inducible NOS in different cells is elicited by diverse signals, including cytokines and microbial products (Nathan 1992). NO is an important mediator of anti-tumor and anti-microbial activity mediated by macrophages and has immunosuppressive effects as well (Hoffman et al. 1993). The cytokine recombinant human interleukin 1 (IL-1) is toxic to rat islet cells in vitro (Mandrup-Poulsen et al. 1986). Since recombinant human IL-1β induces NO production from purified pancreatic beta cells (Corbett et al. 1992) and synergizes with other cytokines to kill cultured pancreatic islet cells (Hamaguchi and Leiter 1990), these cytotoxic effects may, in part, be mediated by increases in intracellular NO concentration. The recent finding of increased NOS mRNA transcripts in macrophage-infiltrated islets of prediabetic BioBreeding (BB) rats but not in islets of diabetes-resistant rats (Kleemann et al. 1993) suggests that NOS associated with inflamed islets is primarily of macrophage origin. However, the finding that purified rat pancreatic beta cells produce NO following incubation with IL-1β (Corbett et al. 1992) suggests that beta cells have the potential to express a cytokine-inducible *Nos* gene. In the present study, we have used the full length (4 kb) mouse macrophage-derived Nos2 cDNA probe (Lyons et al. 1992) to establish the chromosomal location of mouse *Nos2*. This map position was compared with that of a partial (1 kb) cDNA probe from a cytokine-inducible *Nos* gene obtained from neonatal Wistar rat islets (Karlsen et al. 1993) exposed to IL-1β.

Pancreatic islets were isolated from outbred Wistar rat neonates by collagenase digestion (Brunstedt et al. 1984) and preincubated in RPMI 1640 medium with 10% FCS for 4–5 days, then incubated for 24 h in RPMI 1640 medium with 150 pg/ml (60 LAF U/ml) human recombinant IL-1β and 0.5% human serum. RNA was isolated from these islets by guanidinium isothiocyanate treatment and used for poly-A synthesis of cDNA. A PCR product was generated from the cDNA with the primer 5′CCAAGCTTGCCGCCACCATGGCTTGCCCCTGG in conjunction with degenerate primers 3′TG(GA)AACCA(CT)TC(GA)TA(CT)T(TG)(GT)GG(GA)TG (CT)TCCAT spanning bases 256–1254 of the mouse macrophage inducible NOS sequence published by Lyons et al. (Lyons et al. 1992). Sequence similarity is 93% between the rat cDNA product generated and bp 256–1254 of the mouse macrophage inducible Nos sequence (Karlsen, unpublished). Sequence similarities are more than 99% between the rat cDNA product vs. both rat hepatocyte and rat smooth muscle inducible NOS (Geller et al. 1993b; Nunokawa et al. 1993).

The *Nos2* gene was mapped with DNAs isolated from 94 (C57BL/6J × SPRET/Ei)F_1 × SPRET/Ei backcross mice. This backcross panel was provided by The Jackson Laboratory Genetic Mapping Resource and has been characterized for over 600 genetic markers throughout the mouse genome (Rowe et al. 1994). Southern blots of genomic DNA from C57BL/6J (B6) and inbred *Mus spretus* (SPRET/Ei) digested with *Pvu*II were hybridized to the 4-kb mouse macrophage-derived probe. Six fragments were

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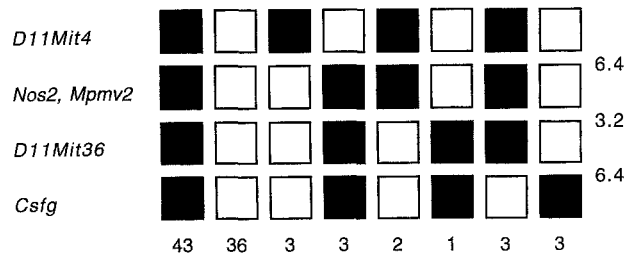

Fig. 1. Segregation of *D11Mit4, Mpmv2, Nos2, D11Mit36,* and *Csfg* on mouse Chr 11 in 94 backcross offspring from the mating (C57BL/6J × SPRET/Ei)F_1 × SPRET/Ei. **Dark squares** represent the C57BL/6J-derived allele, and **open squares** represent the SPRET/Ei-derived allele. The number of progeny carrying each type of chromosome is listed at the bottom, and percentage recombination between pairs of loci is given to the right.

noted for each strain, including a 2.9-kb fragment present in B6 and absent in SPRET/Ei. The 1-kb rat cDNA probe hybridized to four fragments in each strain, including the 2.9-kb fragment present in B6. This 2.9-kb fragment allowed easy differentiation of the B6 versus SPRET/Ei allele in the DNA mapping panel. The typing results (identical for both the 4-kb and the 1-kb probe) were compared with previously mapped polymorphic loci in this DNA panel with the RI manager software (Manly and Elliott 1991). Comparison of haplotype distribution of the *Nos2* gene with other markers previously defined in this backcross panel (Fig. 1) indicated the following locus order and distance (±standard deviation): centromere–*D11Mit4*–6.4 ± 2.5–*Mpmv2/Nos2*–3.2 ± 1.8–*D11Mit36*–6.4 ± 2.5–*Csfg*–telomere. No recombinants were found between *Nos2* and *D11Mit7* (0/90), *D11Mit32* (0/93), *D11Mit34* (0/90), and *Mpmv2* (0/94), in any of the backcross mice typed for these loci. These results position *Nos2* on Chr 11 approximately 46 cM from the centromere.

A locus associated with susceptibility to insulin-dependent diabetes (*Idd4*) in NOD mice has been mapped to Chr 11 within the region delimited by *Acrb* and *D11Nds1* (Ghosh et al. 1993). These markers have been placed 44 and 47 cM respectively from the centromere (GBASE, Oct. 1993) within the same region of Chr 11 with *Nos2*. Destruction of NOD beta cells is associated with a progressively more severe insulitis. Since NO produced by inducible NOS may mediate destruction of beta cells in type 1 diabetes, *Nos2* becomes an attractive candidate for *Idd4*. Macrophages within the leukocytic infiltrates penetrating NOD islets would be expected to be the major source of NO. However, our finding of mRNA present in neonatal rat islets cultured in the presence of IL-1 and apparently encoded by the rat homolog of *Nos2* indicates that the gene is potentially expressible in cytokine-treated islet cells as well as macrophages.

The gene nude (*nu*), sex hormone-binding globulin (*Shbg*), and Avian erythroblastosis viral oncogene homolog 2 (*Erbb2*) have been mapped to mouse Chr 11 between 39 and 56 cM from the centromere. The rat homologs for these genes have been mapped to rat Chr 10 (Cash et al. 1993; Levan et al. 1991). Thus, the rat *Nos2* gene is likely on Chr 10 as well. Similarly, the position of mouse *Nos2* between *D11Mit4* and *Csfg* places it within a segment of mouse Chr 11 that shares linkage homology with human Chr 17. The recent mapping of human NOS2 to human Chr 17p11-17q11 (Charles et al. 1993; Geller et al. 1993a) is in agreement with the position predicted by our mapping results in the mouse.

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