there is no evidence for accommodation in association with a late afterhyperpolarization.

The electrophysiological properties responsible for these firing behaviors are likely to play integral roles in saccade generation and somatic motor acts since mPRF neurons have mono- and poly-synaptic input to neurons of the occulomotor system (*18*) and to lower motoneurons (*19*). For example, in the waking state, the LTS may allow quick induction of action potential firing after the state of relative membrane potential hyperpolarization frequently observed in waking (*20*). The presence of an LTS also suggests that mPRF may play a more complex role in information processing than that of a simple premotor relay. Finally, numerous lines of evidence have implicated mPRF neurons as important in generation of desynchronized sleep events, a time when both the membrane potential is tonically depolarized by 7 to 10 mV and when detailed action potential firing pattern analysis (*21*) indicates the absence of burst discharges. One mechanism for this behavioral state bias toward nonburst discharges may be a change of firing mode for LTS neurons.

REFERENCES AND NOTES

1. E. V. Evarts, *J. Neurophysiol.* **27**, 152 (1964); reviews in M. H. Chase, *Int. Rev. Neurobiol.* **24**, 213 (1983); R. W. McCarley, O. Benoit, G. Barrionuevo, *J. Neurophysiol.* **50**, 798 (1983).
2. R. Llinas and H. Jahnsen, *Nature (London)* **297**, 406 (1982); M. Deschenes, J. P. Roy, M. Steriade, *Brain Res.* **239**, 289 (1982); M. Steriade, M. Deschenes, L. Domich, C. Mulle, *J. Neurophysiol.* **54**, 1473 (1985); M. Steriade, L. Domich, G. Oakson, *J. Neurosci.* 6, 68 (1986); R. W. McCarley *et al.*, in (*1*).
3. K. Ito and R. W. McCarley, *Brain Res.* **292**, 169 (1984).
4. J. A. Hobson, R. W. McCarley, P. W. Wyzinski, *Science* **189**, 55 (1975); R. W. McCarley and J. A. Hobson, *ibid.*, p. 58; R. W. McCarley, J. P. Nelson, J. A. Hobson, *ibid.* **201**, 269 (1978); review in R. W. McCarley, in *The Reticular Formation Revisited*, J. A. Hobson and M. A. B. Brazier, Eds. (Raven, New York, 1980), pp. 375–403.
5. H. L. Haas, B. Scharer, M. Vosmansky, *J. Neurosci. Methods* **1**, 323 (1979).
6. B. P. Fulton and K. J. Walton, *J. Physiol. (London)* **370**, 678 (1986).
7. D. J. Adams, S. J. Smith, S. H. Thompson, *Annu. Rev. Neurosci.* **3**, 141 (1980); J. A. Connor, *Annu. Rev. Physiol.* **47**, 17 (1985).
8. D. V. Madison and R. A. Nicoll, *J. Physiol. (London)* **354**, 319 (1984); P. A. Schwartzkroin and C. E. Stafstrom, *Science* **210**, 1125 (1980); J. R. Hotson and D. A. Prince, *J. Neurophysiol.* **43**, 409 (1980).
9. E. F. Barrett and J. N. Barrett, *J. Physiol. (London)* **255**, 737 (1976); R. Llinas and M. J. Sugimori, *ibid.* **305**, 171 (1980); G. K. Aghajanian, C. P. Vandermaelen, R. Andrade, *Brain Res.* **273**, 237 (1983).
10. R. Llinas and Y. Yarom, *J. Physiol. (London)* **315**, 569 (1981); H. Jahnsen and R. Llinas, *ibid.* **349**, 227 (1984).
11. B. Frankenhaeuser and A. L. Hodgkin, *J. Physiol. (London)* **137**, 218 (1957).
12. S. Hagiwara and L. Byerly, *Annu. Rev. Neurosci.* **4**, 59 (1981).
13. A. Hermann and A. L. F. Gorman, *J. Gen. Physiol.* **78**, 87 (1981); P. R. Stanfield, *Rev. Physiol. Biochem. Exp. Pharmacol.* **97**, 1 (1983).
14. R. W. Aldrich, P. A. Getting, S. H. Thompson, *J. Physiol. (London)* **291**, 531 (1979).
15. A. L. Hodgkin and A. F. Huxley, *ibid.* **116**, 497 (1952).
16. E. Cherubini, R. A. North, A. Surprenant, *Br. J. Pharmacol.* **83**, 3 (1984); J. C. Fowler, R. Greene, D. Weinreich, *J. Physiol. (London)* **365**, 59 (1985); P. Pennefather, B. Lancaster, P. R. Adams, R. A. Nicoll, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 3040 (1985); B. Lancaster and P. R. Adams, *J. Neurophysiol.* **55**, 1268 (1986).
17. S. H. Thompson, *J. Physiol. (London)* **265**, 465 (1977).
18. A. F. Fuchs, C. R. S. Kaneko, C. A. Scudder, *Annu. Rev. Neurosci.* **8**, 307 (1985).
19. B. W. Peterson, *Annu. Rev. Physiol.* **41**, 127 (1979).
20. During waking, mPRF neurons have discharge associated with saccadic eye movements (*17*) and body movements [J. M. Siegel and K. S. Tomaszewski, *J. Neurophysiol.* **50**, 696 (1983); R. P. Vertes, *ibid.* **42**, 214 (1979)].
21. R. W. McCarley and J. A. Hobson, *J. Neurophysiol.* **38**, 751 (1975).

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A Pseudoautosomal Gene in Man

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The X/Y homologous gene *MIC2* was shown to exchange between the sex chromosomes, thus demonstrating that it is a pseudoautosomal gene in man. *MIC2* recombines with the sex-determining gene(s) *TDF* at a frequency of 2 to 3 percent. It is the most proximal pseudoautosomal locus thus far described and as such is an important marker for use in studies directed towards the isolation of *TDF*.

HALF A CENTURY AGO KOLLER AND Darlington observed partial meiotic pairing of sex chromosomes in male rats and proposed that recombination might occur between the morphologically distinct sex chromosomes (*1*). If genes are exchanged between the sex chromosomes they would not show classical sex linkage and the descriptive term "pseudoautosomal" was proposed for such loci (*2*). In the mouse, the abnormal *Sxr* locus is pseudoautosomal and the gene *STS* may also exchange between the X and Y chromosomes (*3*). In man, however, *Sts* is not a pseudoautosomal gene (*4*) and a search for pseudoautosomal disease loci proved unsuccessful (*5*). Recent molecular analysis of the human Y chromosome has shown that in man a pseudoautosomal region does exist (*6*). In this report we describe a human pseudoautosomal gene and present evidence that it is also the closest known marker distal to the sex-determining gene(s) (*TDF*) in man.

The monoclonal antibody 12E7 recognizes a cell surface antigen that is encoded by the *MIC2* gene (*7*). By means of somatic cell genetics, *MIC2* genes were found on both human sex chromosomes (*7*). Biochemical analysis suggested that the products of the two genes are very closely related or identical (*8*) and subsequent isolation and analysis of a complementary DNA (cDNA) clone, corresponding to an *MIC2* gene, pSG1, confirmed the close relationship between the X- and Y-located *MIC2* genes at the DNA level (*9*). By means of pSG1 as a probe for in situ hybridization, the *MIC2* genes were localized to the short arm of the Y chromosome and the tip of the short arm of the X chromosomes (*10*). This localization was consistent with *MIC2* being pseudoautosomal in nature (*2, 6*). Several different blocks of homologous sequences are, however, shared between the human sex chromosomes, in addition to those in the pseudoautosomal region (*11*) and formal identification of the *MIC2* locus as pseudoautosomal required demonstration of exchange between the X and Y chromosomes during male meiosis.

Like several other pseudoautosomal sequences that have been described (*6*), pSG1 detects multiple restriction fragment length polymorphisms (RFLP's). The 1.0-kb pSG1 sequence detects a number of apparently independent RFLP's in genomic DNA's that have been digested with Taq I, Msp I, Hind III, Pvu II, and Pst I. The pattern of hybridization observed in Taq I– (Fig. 1a), Hind III–, Pvu II– and Pst I–digested DNA's was very complex with several constant (C) and polymorphic (V) bands of varying intensity, while in Msp I digestions a simple two-allele system was revealed (Fig. 1b, a_1 and a_2). The details of the RFLP's detected by pSG1 will be described (*12*). The extensive variability detected at the *MIC2* locus by pSG1 should make the cloned sequence a highly informative marker for use in family studies; however, because of the complexity of the pattern of constant

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Table 1. Segregation of *MIC2*, *DXYS17*, *DXYS15*, and *DXYS14* with respect to *TDF* and to one another. Combining these data with those of Rouyer *et al.* (*6*), distances between *MIC2*, *DXYS17*, *DXYS15*, and *DXYS14* and the sex-determining gene(s) are estimated as follows [$\hat{\theta}$, maximum likelihood estimate of distance (*15*)]: *MIC2* and *TDF*, $\hat{\theta} = 0.02$, confidence interval = (0.00; 0.09); *DXYS17* and *TDF*, $\hat{\theta} = 0.16$, confidence interval = (0.09; 0.25); *DXYS15* and *TDF*, $\hat{\theta} = 0.33$, confidence interval = (0.20; 0.50); *DXYS14* and *TDF*, $\hat{\theta} = 0.47$, confidence interval = (0.36; 0.50). In this study Taq I genotypes for the *MIC2* pSG1 and p19B clones were determined as well as the MspI pSG1 genotypes for one family. Taq I genotypes were determined for *DXYS17* (probe 601), *DXYS15* (probe 113D), and *DXYS14* (probe 29C1). The RFLP's detected were as described (*6*) and examples of the alleles observed are shown in Fig. 2. There were very few meioses informative for *DXYS15* (probe 113D).

Analysis	Loci									
	MIC2 and *TDF*	*DXYS17* and *TDF*	*DXYS15* and *TDF*	*DXYS14* and *TDF*	*MIC2* and *DXYS17*	*MIC2* and *DXYS15*	*MIC2* and *DXYS14*	*DXYS17* and *DXYS15*	*DXYS17* and *DXYS14*	*DXYS15* and *DXYS14*
Recombination events	1	7	3	14	6	1	12	2	8	0
Informative male meioses	46	38	7	34	34	3	33	4	31	7
Recombinant sex chromosomes (%)	2.2	18.4	42.9	41.2	17.6	33.3	36.4	50.0	25.8	0.0

and polymorphic bands observed with most enzymes, the interpretation of inheritance of the various alleles is difficult. The problem was circumvented by isolating genomic sequences that detected simple patterns in Southern blot analysis. An *MIC2* genomic sequence, p19B, subcloned from a λ phage recombinant that hybridized with pSG1, was found to detect RFLP's in Taq I and Pvu II digestions (*13*). Both the Taq I and Pvu II polymorphisms detected by p19B are apparently due to simple mutations in the enzyme recognition sequence with which they are detected (*13*).

The inheritance of the *MIC2* RFLP's was investigated in three large kindreds (*14*). Individuals were genotyped for the Taq I RFLP's detected by pSG1 and p19B as shown (Fig. 2, a and b). The pSG1 Msp I genotypes were also determined in one of the families. A total of 46 informative male meioses were analyzed (Table 1) and a single recombinant between *MIC2* and *TDF* was observed. The individual who is a recombinant between *MIC2* and *TDF* (Fig. 2b, lane 5, individual II-4) is a normal, fertile female. A Southern blot analysis of the family in which the recombinant with *MIC2* was discovered is present in Fig. 2 and a simplified pedigree illustrating inheritance of p19B is shown in Fig. 3. We conclude that *MIC2* is an expressed pseudoautosomal gene.

The low frequency with which *MIC2* recombines with *TDF* is in marked contrast to the frequencies observed for those pseudoautosomal sequences described previously (*6*). *DXYS14, DXYS15*, and *DXYS17* have been reported to exchange between the X and Y chromosomes at frequencies of 49.5, 31.5, and 14%, respectively (*6*). The low rate of exchange (1/46 meioses) at the *MIC2* locus suggests it is much closer to the locus or loci for sex determination than any of the other pseudoautosomal sequences. This was confirmed by testing for the segregation of Taq I RFLP's that are detected by *DXYS17, DXYS15*, and *DXYS14* in the families studied for the inheritance of *MIC2*. The RFLP's observed were as described (*6*) and the pattern of segregation of the sequences in one family is illustrated in Fig. 2. Table 1

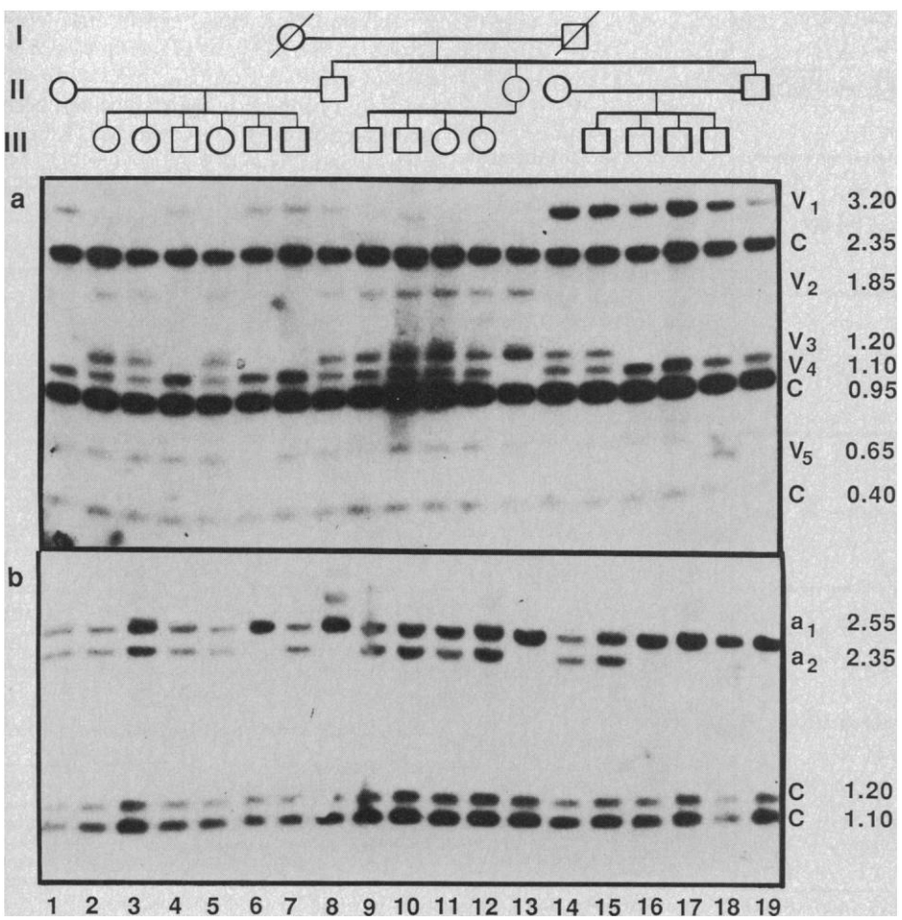

Fig. 1. Southern blot analysis of family member DNA's hybridized with pSG1. Ten-microgram samples of DNA were digested to completion with a fourfold excess of enzyme (Anglia or Bethesda Research Laboratories). The digested DNA's were size-fractionated by electrophoresis through 0.8% agarose gels and transferred to GeneScreen Plus membrane (New England Nuclear). Prehybridization and hybridization of ^{32}P-labeled random primed (*17*) pSG1 were as described by New England Nuclear. Blots were washed twice at room temperature in 2× SSC (standard saline citrate) and then twice in 0.2× SSC, 0.2% sodium dodecyl sulfate at 65°C. Autoradiography was carried out at −70°C for 16 to 96 hours (Kodak XAR film with Dupont Lightning-Plus intensifying screens). (a) Taq I digestions. Invariant bands are marked C and variable bands are marked V. V_3 and V_4 represent allelic forms. (b) Msp I digestions. The allelic fragments are indicated as a_1 and a_2. Constant bands are marked C. Relationships between the individuals for whom DNA samples were analyzed are indicated by the pedigree above. The complex pattern of hybridization in Taq I digestions makes analysis difficult. Fragment sizes (kilobases) based on Hind III–digested λ markers are given.

provides a summary of results for recombination between the pseudoautosomal loci and *TDF* and with respect to one another. Recombination frequencies between *TDF* and both *DXYS17* and *DXYS14* were similar to those reported previously (*6*). The rates of recombination with *DXYS15* could not be estimated accurately as there were too few informative matings (Table 1). Combining the data of Rouyer *et al.* (*6*) with those from this study, maximum likelihood estimates for the distances between *MIC2, DXYS17, DXYS15*, and *DXYS14* and the sex-determining locus/loci were calculated (*15*). Those estimates (Table 1) indicate that *MIC2* is significantly closer to *TDF* than the other three pseudoautosomal loci. The recombination event we observed between *MIC2* and *TDF* (Fig. 2b, lane 5, individual II-4) appears to be the result of a single crossover event in which the paternal Y-linked pseudoautosomal sequences have exchanged with the X chromosome. Individual II-4 is also a recombinant with respect to *DXYS17* and sex, the only such recombinant in the sibship of 8 (Fig. 2c). Because the paternal grandparents were not analyzed, we cannot be absolutely certain as to which alleles detected by *DXYS14* (Fig. 2e, probe 29C1) are on the Y chromosome and which are on the X chromosome in individual I-1 (lane 1). Assuming that a double crossover event between the X and Y chromosomes took place in the *MIC2/TDF* recombination event, then the genetic distances between *MIC2* and *DXYS14* and between *DXYS17* and *DXYS14* would increase. Given the pattern of segregation seen in the family shown in Fig. 2, it is unlikely that a double recombination event took place.

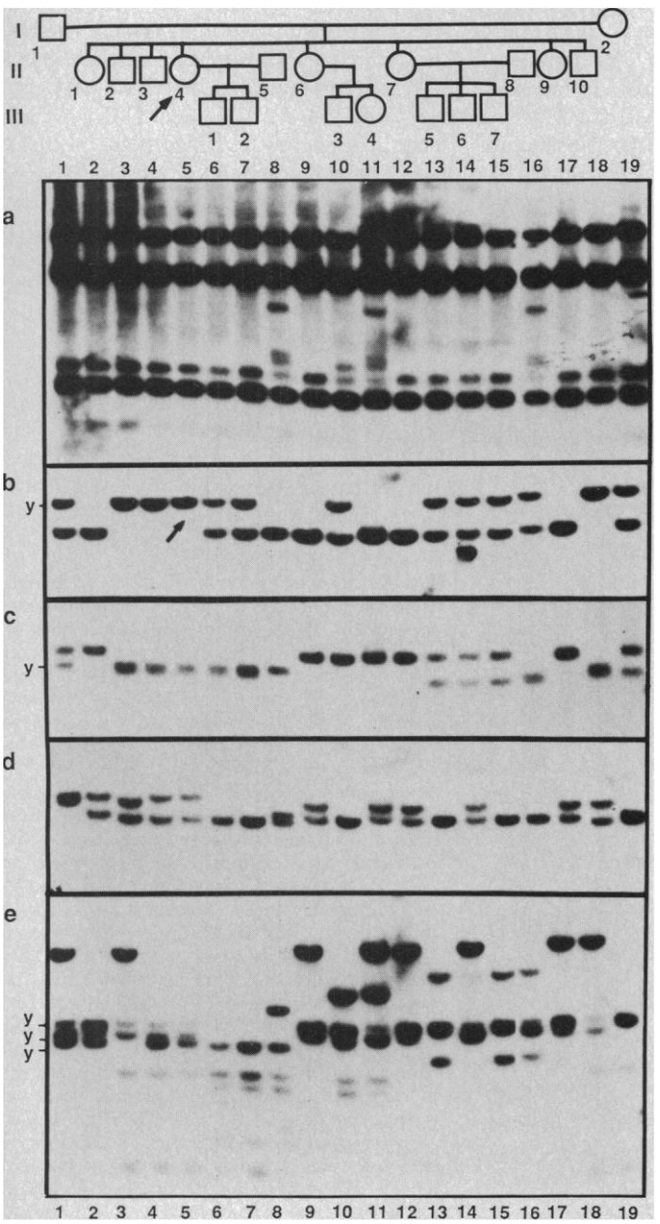

Fig. 2. Southern blot analysis of TaqI-digested family member DNA's in which a recombination between *MIC2* and *TDF* was observed. The relationships between individuals studied is indicated by the pedigree above. Sequential hybridizations were as follows: (a) pSG1 (*MIC2* cDNA), (b) p19B (*MIC2* genomic sequence), (c) *DXYS17* (probe 601), (d) *DXYS15* (probe 113D), and (e) *DXYS14* (probe 29C1). The individual who is a recombinant for *MIC2* and sex phenotype (II-4, lane 5) is indicated with an arrow. Note that pSG1 and *DXYS15* (probe 113D) are largely uninformative in this family. The Y alleles in I-1 (lane 1), in whom the recombination event took place, are all marked with Y. Taq I alleles detected by p19B are 2.5 kb and 3.2 kb. □, male; ○, female.

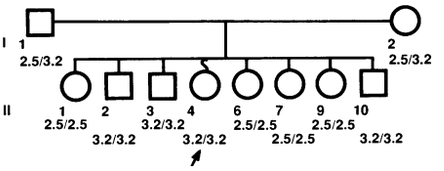

Fig. 3. Inheritance of *MIC2* in the family in which recombination with *TDF* occurred. Taq I genotypes at the *MIC2* locus were determined with the genomic probe p19B (as illustrated in Fig. 2b). The 2.5-kb allele is carried on the paternal X chromosome while the 3.2-kb allele is on the Y chromosome. All sons inherit the Y-linked 3.2-kb allele from their father, and all daughters (with the exception of the recombinant II-4; ↗) inherit the 2.5-kb allele from their father. The arrow indicates the recombinant. 2.5 and 3.2 refer to the sizes of Taq I alleles (kb) detected by p19B. □, male; ○, female.

The analysis of DNA from human XX males (phenotypic males with an XX or female karyotype) has shown that approximately two-thirds of all such individuals inherit sequences derived from the Y chromosome (*16*). If it is assumed that XX males result from a single abnormal exchange between the sex chromosomes, then it follows that they must have an intact pseudoautosomal region and a limited amount of Y chromosome–specific material including the gene(s) responsible for sex determination. The order of Y-specific sequences proximal to the sex-determining gene(s) has been determined on the basis of their presence or absence in DNA from XX males (*16*) and the order of pseudoautosomal loci has been established through family studies [Rouyer *et al.* (*6*) and this study]. At present *MIC2* is the closest flanking marker known to be distal to the sex-determining gene(s).

REFERENCES AND NOTES

1. P. C. Koller and C. D. Darlington, *J. Genet.* **29**, 159 (1934).
2. P. S. Burgoyne, *Hum. Genet.* **61**, 85 (1982).
3. L. Singh and K. W. Jones, *Cell* **28**, 205 (1982); E. P. Evans, M. D. Burtenshaw, B. M. Cattanach, *Nature (London)* **300**, 443 (1982); E. Keitges, M. Rivest, M. Siniscalco, S. M. Gartler, *ibid.* **315**, 226 (1985).
4. T. Mohandas, L. J. Shapiro, R. S. Sparkes, M. C. Sparkes, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 5779 (1979); L. Tiepolo *et al.*, *Hum. Genet.* **54**, 205 (1980).
5. J. B. S. Haldane, *Ann. Eugen.* **7**, 28 (1936).
6. M.-C. Simmler *et al.*, *Nature (London)* **317**, 692 (1985); H. J. Cooke, W. R. A. Brown, G. A. Rappold, *ibid.*, p. 687; F. Rouyer *et al.*, *ibid.* **319**, 291 (1986).
7. P. N. Goodfellow, G. Banting, R. Levy, S. Povey, A. McMichael, *Somatic Cell Genet.* **6**, 777 (1980); P. N. Goodfellow *et al.*, *Nature (London)* **302**, 346 (1983).
8. G. S. Banting, B. Pym, P. N. Goodfellow, *EMBO J.* **4**, 1967 (1985).
9. S. M. Darling, G. S. Banting, B. Pym, J. Wolfe, P. N. Goodfellow, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 135 (1986).
10. V. Buckle, C. Mondello, S. Darling, I. W. Craig, P. N. Goodfellow, *Nature (London)* **317**, 739 (1985).
11. P. N. Goodfellow, S. Darling, J. Wolfe, *J. Med. Genet.* **22**, 329 (1985).
12. P. J. Goodfellow *et al.*, in preparation.

13. The p19B subclone is a unique 1.0-kb Eco RI intron sequence. Its identity was confirmed by mapping it to the regions Xp22.32-pter and Yq.1-pter by hybridizations to somatic cell hybrid DNA. The restriction map of the cloned sequence p19B and adjacent cloned sequences are consistent with the Taq I and Pvu II RFLP's being due to simple mutations in the enzyme recognition sites.
14. One family included in this study has multiple endocrine neoplasia type 2A. Another family (BD10) segregates Becker muscular dystrophy and the third (DEX1) segregates Duchenne muscular dystrophy.
15. Lod scores [as described by Morton, *Am. J. Hum. Genet.* 7, 277 (1955)] were calculated to determine maximum likelihood estimates for distances between the pseudoautosomal loci and the *TDF*. The confidence intervals were estimated by taking the θ values (recombination fractions) at the maximum lod score ($\hat{z}$) minus 1, as recommended by Conneally *et al.* [*Cytogenet. Cell Genet.* **40**, 356 (1985)].
16. G. Guellaen *et al.*, *Nature (London)* **307**, 172 (1984); G. Vergnaud *et al.*, *Am. J. Hum. Genet.* **38**, 109 (1986).
17. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **137**, 266 (1984).
18. We thank our colleagues G. Banting, B. Pym, C. Pritchard, M. V. Wiles, and P. Harper for discussion and comments on this manuscript; C. Middlemiss is gratefully acknowledged for her expert secretarial support.

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Pertussis Toxin Inhibition of B Cell and Macrophage Responses to Bacterial Lipopolysaccharide

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Lipopolysaccharide, a component of the outer membrane of Gram-negative bacteria, activates B lymphocytes and macrophages. Pertussis toxin, which inactivates several members of the G protein family of signaling components, including G_i and transducin, was found to inhibit the lipopolysaccharide-induced responses of the WEHI-231 B lymphoma cell line and the P388D$_1$ macrophage cell line. These results, combined with the demonstration that lipopolysaccharide inhibits adenylate cyclase activity in P388D$_1$ cells, strongly argues that lipopolysaccharide activation of cells is mediated by a G_i-like receptor-effector coupling protein.

BACTERIAL LIPOPOLYSACCHARIDE (LPS) is a potent activator of both B lymphocytes and macrophages. Lipopolysaccharide accelerates the phenotypic maturation of pre-B cells and immature B cells to the mature B-cell stage and induces mature B cells to proliferate and differentiate into antibody-secreting plasma cells (*1*). In addition, LPS induces macrophages to secrete immunoregulatory substances including interleukin-1 (IL-1), interferons α and β, and prostaglandins. In conjunction with interferon-γ, LPS induces macrophages to become tumoricidal and bactericidal (*2*). Thus, LPS induces a complicated and extensive set of host immune reactions. Other components of the bacterial cell wall, including peptidoglycan, certain outer-membrane proteins, and exopolysaccharides, have similar properties (*3*). Thus, it is attractive to consider the possibility that higher organisms have evolved mechanisms that enable the immune system to recognize bacterial products, thereby contributing to rapid and vigorous immune responses to bacterial infections.

Little is known about the molecular details by which these bacterial products stimulate B cells and macrophages. We recently showed that LPS can activate the receptor coupling component G_i, which inhibits adenylate cyclase (*4*), in two LPS-responsive murine cell lines: WEHI-231, an immature B-lymphoma cell line, and P388D$_1$, a macrophage cell line (*5*). LPS inhibited adenylate cyclase in membranes from P388D$_1$ cells, and, as in other systems in which hormones or other regulatory molecules inhibit adenylate cyclase, this inhibition was abolished by first treating the cells with pertussis toxin, which inactivates G_i by ADP-ribosylating (ADP, adenosine diphosphate) the α subunit of G_i (*4*). Furthermore, it was observed that pretreatment of the membranes from P388D$_1$ or WEHI-231 cells with LPS and GppNHp, a nonhydrolyzable analog of guanosine triphosphate (GTP), abolished the ADP-ribosylation of G_i by pertussis toxin in vitro (*5*). Because only the inactive form of G_i is a substrate for pertussis toxin, this result suggests that LPS, through its putative receptor, can activate G_i. Because these biochemical experiments indicated that LPS can activate G_i in isolated membranes, we used pertussis toxin to determine whether G_i activation is important for the biological responses of these cells to LPS.

Upon appropriate stimulation, normal macrophages rapidly accumulate a membrane-associated form of IL-1 (mIL-1) before release of soluble IL-1 (*6*). Similarly, the P388D$_1$ cell line rapidly made mIL-1 upon stimulation with LPS (Fig. 1). Incubation of P388D$_1$ cells with LPS for as little as 15 minutes induced a measurable increase in the amount of mIL-1 above the basal level found in unstimulated cells. The amount of mIL-1 was increased appreciably within 2 hours of stimulation with LPS and was increased nearly 40-fold after 24 hours.

If the activation of G_i by LPS observed in biochemical experiments reflects a necessary event for the biological response to LPS, then treatment with pertussis toxin should inhibit mIL-1 production. P388D$_1$ cells were cultured with pertussis toxin for 4 hours and then stimulated with LPS for an additional 2 hours in the presence of the toxin. This toxin treatment totally inhibited the LPS-induced increase in mIL-1 (Fig. 2). In addition, the level of mIL-1 present in unstimulated cells was lowered somewhat. Under these conditions pertussis toxin was a very potent inhibitor of the LPS response; pertussis toxin treatment at only 0.3 ng/ml

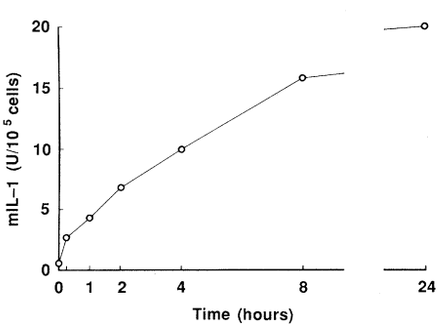

Fig. 1. LPS induction of mIL-1 accumulation in P388D$_1$ cells. P388D$_1$ cells (10^6 per milliliter in RPMI 1640 medium containing 10% fetal bovine serum) were cultured with LPS (5 μg/ml) (from *Escherichia coli* 0111:B4; Difco) for the indicated time. The cells were washed twice, fixed with paraformaldehyde (1% in medium for 15 minutes at room temperature), washed twice more, and cultured overnight in medium at 37°C. The IL-1 content of the cells was determined in the thymocyte comitogenesis assay: graded numbers of fixed cells in 0.2 ml were cultured with 10^6 thymocytes from C3H/HeJ mice in the presence of phytohemagglutinin (PHA) (3 μg/ml; Vector Laboratories, Burlingame, CA). After 72 hours of culture, [^{3}H]thymidine (1 μCi per well, 6.7 Ci/mmol; ICN, Irvine, CA) was added, and 4 hours later the cultures were harvested with an automated cell harvester. Incorporation of [^{3}H]thymidine was measured by liquid scintillation counting. One unit of IL-1 was defined as the amount of material required to induce one-third maximal proliferation of the thymocytes stimulated with PHA + IL-1. The error in unit determination was estimated to be less than 25% of the value for each determination reported.

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