

is inability of the mutant chain to participate in trimer assembly, leading to a 50% reduction in collagen X. We suggest that the mechanism of growth plate abnormalities in Schmid MCD is similar to that in the mice. The transgenic mice described here will, therefore, not only provide insights into collagen X regulation and function during EO, but can also serve as models for chondrodysplasias with spondylometaphyseal involvement. □

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Molecular characterization of a peripheral receptor for cannabinoids

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THE major active ingredient of marijuana, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), has been used as a psychoactive agent for thousands of years. Marijuana, and Δ^9 -THC, also exert a wide range of other effects including analgesia, anti-inflammation, immunosuppression, anticonvulsion, alleviation of intraocular pressure in glaucoma, and attenuation of vomiting¹. The clinical application of cannabinoids has, however, been limited by their psychoactive effects, and this has led to interest in the biochemical bases of their action. Progress stemmed initially from the synthesis of potent derivatives of Δ^9 -THC^{4,5}, and more recently from the cloning of a gene encoding a G-protein-coupled receptor for cannabinoids⁶. This receptor is expressed in the brain but not in the periphery, except for a low level in testes. It has been proposed that the non-psychoactive effects of cannabinoids are either mediated centrally or through direct interaction with other, non-receptor proteins^{1,7,8}. Here we report the cloning of a receptor for cannabinoids that is not expressed in the brain but rather in macrophages in the marginal zone of spleen.

To identify novel G-protein-coupled receptors expressed in myeloid cells, polymerase chain reaction (PCR) using degenerate primers was done on complementary DNA prepared from the human promyelocytic leukaemic line HL60. Treatment of HL60

cells with dimethylformamide (DMF) induces granulocyte differentiation, whereas tetradecanoylphorbol acetate (TPA) induces macrophage differentiation⁹. Amplification products from DMF-treated cells were cloned and sequenced, and six classes of clone showed homology to the G-protein-coupled receptor family. Two of these classes corresponded to previously identified receptors; the interleukin-8 receptor-B (ref. 10) and the adenosine A3 receptor¹¹ (S.M., manuscript in preparation). Of the remaining four sequences, only one showed particular homology to a published receptor. This clone, CX5, was related to a cannabinoid receptor cloned originally from rat brain⁶. To investigate the functional significance of this homology, the CX5 insert was used to screen an HL60 cDNA library. Two cDNA clones were obtained, hCX5.1 and hCX5.36, the latter extending the furthest 5' and the complete nucleotide sequence of this clone is shown in Fig. 1a. The protein encoded by hCX5.36 shows 44% identity with the human cannabinoid receptor and the degree of identity rises to 68% for those transmembrane residues proposed to confer ligand specificity¹².

To determine if the CX5 receptor binds cannabinoids, the hCX5.36 cDNA was inserted into an expression vector and transfected into tissue culture cells. Figure 2a shows a binding curve of the cannabinoid receptor ligand Win 55212-2 (ref. 13) to membranes prepared from the transfected cells. The control cells do not express receptors for cannabinoids, but expression of hCX5.36 causes the appearance of a saturable number of high-affinity binding sites for WIN 55212-2 and also for a second high-affinity cannabinoid CP55,940 (ref. 5; Fig. 2b, and data not shown). The affinities of the receptor for these structurally unrelated ligands (Win 55212-2: dissociation constant K_d 3.7 nM (± 0.4 nM); CP55,940: K_d 1.6 nM (± 0.5 nM)), are comparable to the analogous figures (24 nM and 2–15 nM) reported for the brain receptor^{13,15}. Furthermore, competition binding analysis showed that the CX5 receptor can distinguish between closely related derivatives of the archetypal cannabinoid Δ^9 -THC, showing a lower affinity for the relatively inactive cannabidiol, than for the biologically active Δ^9 -THC, cannabinol and 11-OH- Δ^9 -THC (Fig. 2b). Thus it appears that hCX5.36 encodes a selective, high-affinity receptor for cannabinoids. Note that although cannabidiol is only weakly cannabimimetic and binds the brain receptor with an affinity about 10-fold less than that of Δ^9 -THC^{5,14}, this ligand binds to the CX5 receptor with an affinity comparable to that of Δ^9 -THC (250 nM versus 320 nM, Fig. 2b). This suggests that cannabidiol may have a preference for the CX5 receptor over the brain receptor. Recently, a novel compound isolated from brain, arachidonylethanolamide (anandamide), has been identified as a candidate ligand for the brain receptor¹⁶ and Fig. 2b shows that this compound can also bind to the CX5 receptor. The binding affinity (K_i 1.6 μ M (± 0.4)) is lower than that reported for brain membranes (K_i 52 nM), but it should be noted that the apparent receptor affinities of cannabinoids can vary depending on the assay system used^{5,14,15}. In the following text we shall refer to the original receptor as CB-R and this new receptor as CX5.

When CX5 was used to probe northern blots of RNA from HL60 cells it hybridized to two transcripts of about 2.5 and 5.0 kilobases (kb) (Fig. 3a). The two transcripts probably arise from the use of alternative poly(A)⁺ addition sites. Clone hCX5.1 does not have a poly(A)⁺ tail at the position of that in hCX5.36, but instead extends further in the 3' direction (Fig. 1a). The putative polyadenylation sequence of hCX5.36 (GAUAAA) is a variant of the AAUAAA consensus that is found in a small fraction of messages and which can be used, albeit inefficiently, *in vitro*¹⁷. CX5 is expressed in uninduced HL60 cells, but transcript levels are elevated further on myeloid, or granulocyte, differentiation, although the gene does not appear to be expressed in mature neutrophils isolated from blood (data not shown).

To investigate the tissue distribution of CX5, a portion of a rat homologue was isolated by PCR. This rat probe (rCX5) detects an mRNA of about 2.5 kb in spleen, but not in a variety

FIG. 1 Nucleotide and protein sequences of the cDNAs hCX5.36 and hCX5.1. *a*, Nucleotide sequence of the hCX5.36 cDNA and the protein sequence encoded by the longest open reading frame, the in-frame stop codon upstream of the most 5' ATG is underlined. Also shown is the nucleotide sequence of hCX5.1 where it diverges from that of hCX5.36 after the putative poly(A) addition site (underlined). *b*, Comparison of the protein encoded by hCX5.36 with the previously reported human cannabinoid receptor²⁸. Identities are boxed and the seven putative transmembrane segments are underlined. METHODS. Oligo-dT primed cDNA was synthesized from poly(A)⁺ RNA prepared from HL60 cells induced with 0.5% DMF for 3 days. cDNA (5 ng in 20 µl) was amplified with *Taq* polymerase using degenerate primers encoding regions conserved between many G-protein-coupled receptors. Those that produced CX5 were GAGGGCCATYISNNTNGAYMGNTA and TGAAGCTTSHRTANANSANNGGRTT (encoded regions in bold in sequence alignment). 40 cycles of 94 °C, 1 min, 50 °C, 2 min and 72 °C 2 min, in 10 mM Tris-HCl pH 8.3, 3 mM MgCl₂, 100 mM tetramethylammonium chloride, 0.05% Tween 20, 0.05% NP-40, 250 µM dNTPs, 20 µM each primer. Gel-purified amplification products were digested with *Apal* and *HindIII* and cloned into Bluescript. After classification of the products by sequencing, the insert from clone CX5 was used to screen 2 × 10⁵ colonies of a cDNA library from TPA-treated HL60 cells²⁹ (from D. Simmons). Both hCX5.1 and hCX5.36 were isolated several times and after restriction mapping and partial sequencing of both clones, the complete nucleotide sequence of hCX5.36 was determined by primer walking using double-stranded dideoxy-sequencing. The GenBank accession number for hCX5.36 (CB2) is X74328.

b

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hCB-R      MKSILDGLADTTFTITITDLYVGSNDIQYEDIKGDMSKLGYPFQ
hCX5        MEECWVTEIANGSKDGLDSN --- 20
hCB-R      KFPLTSFRGSPFOEKMTAGDNQPLVPADQVNIIEFYNSLSFKENEI
-----PMKDYMTLSGPKTAVAVCTLEQLLSALENVAVLYLTSSHO
OQGENFMDETCFIMNPSQOLATAVLSLTGTFVLENLLVCVILHSRS
113
LRRKPSYFIGSLAGADPDSVAFSPVNFHFGVDSKAVFLRIGSM
LRCRPSYFIGSLAGADLVSVITVYSHIDHVFHRSRNVFLRIGSM
113
IMFTASVGSLLTAIDRYLCLRYPSYVALLTRGRGLITLGMVLSAL
163
TASEASVGSLLTAIDRYLTSIHRELAKYRIVTEPKAMAFCLMTIATV
163
VSYLPDGNITCPRP--CSELPFLPNQVLSLALLFIAHFSGLITVIGH
211
IAVLPDGNITCPRP--CSELPFLPNQVLSLALLFIAHFSGLITVIGH
211
VLWKAHRAVASL-----SGHQDROV--FGMARMLDMLAKT
246
ILWKAHRAVARMIRGRTQKSIITHTSEDKGVQVTRDQARM--DILAKT
246
LGLMDVLLTQVFLMAHSLATLSDQVKAFAFCSMLCLINSMVNP
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LMLTLLVLLTQVFLMAHSLATLSDQVKAFAFCSMLCLINSMVNP
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IIYALRSKDLHFAFRSMFPSCGTAQPLDMSMGDSCLHGHANNAASVHR
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IAESCISKSTV-----KIAKVTMSVSTDTSAEL
360
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a

HCX5.36

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CAGTCTCTGGGAGAGGACAGAAAACTGGACTCTCAGCCCCGGGAGCTCCAGTGCCAGCCACCCACACACAAACCCAAAGCCTT 90
MetGluGluCysTrpValThrGluIleAlaAsnGlySerLysAspGlyLeuAsp
CTAGACAAGCTCAGTGAATCTGAGGGCCACCCCATGGAGGAATGCTGGTGACAGAGATAGCCAATGGCTCCAGGATGGCTGGAT 180
SerAsnProMetLysAspTyrMetIleLeuSerGlyProGlnLysThrAlaValAlaValLeuCysThrLeuLeuGlyLeuLeuSerAla
TCCAACCCCTATGAAGGATTACATGATCTCAGAGCTGTCAGGAGAGAGCTGTTGCTGTGTGTGCACTCTCTGGGCTGCTTAAGTGCC 270
LeuGluAsnValAlaValLeuTyrLeuIleLeuSerSerHisGlnLeuArgArgLysProSerTyrLeuPheIleGlySerLeuAlaGly
CTGGAGAACCTGGCTGTGCTCTATCTGATCTGCTCCACCACTCCGCGGAGGCTCATACCTGTTCTATTGGCAGCTTGGCTGGG 360
AlaAspPheLeuAlaSerValValPheAlaCysSerPheValAlaAsnPheHisValPheHisGlyValAspSerLysAlaValPheLeuLeu
GCTGACTTCTGCGGAGTGTGCTTTTGTGCTGAGCTTTGTGAATTTCCATGTTTCCATGGTGGATTCCAAGGCTGTCTTCTGCTG 450
LysIleGlySerValThrMetThrPheThrAlaSerValGlySerLeuLeuLeuThrAlaIleAspArgTyrLeuCysLeuArgTyrPro
AAGATTGGCAGCTGACTATGACTTTCACAGCTCTGTGGTACCTCTGCTGACCGCATGACCGATACCTCTGGCTGCTATCCA 540
ProSerTyrLysAlaLeuLeuThrArgGlyArgGlyLeuValThrLeuGlyIleMetTrpValLeuSerAlaLeuValSerTyrLeuPro
CCTTCTCAAGCTCTGCTCACCCTGAGGAGGAGTGTGGTACCTCTGCTGACCGCATGACCGATACCTCTGGCTGCTATCCA 630
LeuMetGlyTrpThrCysCysProArgProCysSerGluLeuPheProLeuIleProAsnAspTyrLeuLeuSerTrpLeuLeuPheIle
CTCATGGTGGACTTGTCTGCCAGGCTGTCTCTCCACTGTTTCCACTGATCCCAATGACTACCTGCTGAGCTGGCTGCTGTTCTATC 720
AlaPheLeuPheSerGlyIleIleTyrThrTyrGlyHisValLeuTrpLysAlaHisGlnHisValAlaSerLeuSerGlyHisGlnAsp
GCCTTCTCTTTTCCGGAATCATCTACACCTATGGCATGTCTCTGGAAGGCCCATCAGCATGTGGCCAGCTTGTCTGGCCACAGGAC 810
ArgGlnValProGlyMetAlaArgMetArgLeuAspValArgLeuAlaLysThrLeuGlyLeuValLeuAlaValLeuLeuIleCysTrp
AGGCAGGTGCCAGGAATGGCCGAATGAGGCTGGATGTGAGGTGGCCAGACCCATAGGCTAGTGTGGCTGTGCTCTCATCTGTTGG 900
PheProValLeuAlaLeuMetAlaHisSerLeuAlaThrThrLeuSerAspGlnValLysLysAlaPheAlaPheCysSerMetLeuCys
TTCCAGTGTGCTGCTCATGGCCACAGCTGGCCACTACGCTCAGTACCAGGTCAAGAGGCCCTTGTCTTCTGCTCCATGCTGTGC 990
LeuIleAsnSerMetValAsnProValIleTyrAlaLeuArgSerGlyGluIleArgSerSerAlaHisHisCysLeuAlaHisTrpLys
CTCATCACTCCATGGTCAACCTGTCTATGCTCTACGGAGTGGAGAGATCCGCTCTCTGCCCATCAGTCCCTGGCTGCTGCTGAGG 1080
LysCysValArgGlyLeuGlySerGluAlaLysGluGluAlaProArgSerSerValThrGluThrGluAlaAspGlyLysIleThrPro
AAGTGTGTGAGGGGCTTGGGTGAGGCAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG 1170
TrpProAspSerArgAspLeuAspLeuSerAspCys***
TGGCAGATTCAGAGATCTAGACCTCTCTGATTGCTGATGAGGCTCTTCCCAATTTAAACAACCTCAAGTCAGAAATCAGTTCCTCC 1260
TGGAGAGAGAGAGAGGGTCTTGGCACTCTCTTACTTAAACAGTCCAGACACCTAGACACGAGCCCTTTTGTCTGATGAGTGTG 1350
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CCAGGCGGCAATGAGGAGCTTGGGAGAAATCTGAGAAGATGGGTGTCTCTTGGGAAGTCAGGATATCAGATGGATGGACATCCA 1620
GGTCTTCTCTGCTGCTAATTGTCAAGGCTCTTGGCTCTGGAGCTATGAAAGGCCCATTTCAAGTCACCCCTTGGCACTGAGGACCGA 1710
GGACTATGCTATGATGAGGATTAAGGTGTGACTTGCTCTTTTCCAGATATAATGACAAGCCTTCAAAAAAAAAAAAAA 1790
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HCX5.1:

....TATGCTATGATGAGGATTAAGGTGTGACTTGCTCTTTTCCAGATATAATGACAAGCCTTCAGTTTGGGTCATCTGTTGTTTG.

of other tissues (Fig. 3b). In particular, the rCX5 transcript is not detected in brain, even though the 6 kb mRNA encoding the rat CB-R can be readily detected in the same sample. The expression pattern of the CB-R gene corresponds well to the distribution of binding sites for cannabinoids in the brain^{6,18}. But it is possible that CX5 is expressed in a subset of these sites and its expression level is too low to be detected in total brain RNA. To investigate this possibility horizontal sections of rat brain were probed by *in situ* hybridization with labelled oligonucleotides corresponding to rat CB-R and to rCX5 (Fig. 4). As previously reported, the brain receptor has a widespread distribution with high levels of expression in the cortex, hippocampus, striatum and cerebellum. When adjacent sections were probed for rCX5, no expression could be detected in these, or any other, regions. The rCX5 oligonucleotide does, however, hybridize to localized regions of the spleen (Fig. 4b, c). The expression appears concentrated in the marginal zones found around the periarteriolar lymphoid sheaths. The expression of hCX5 in HL60 cells differentiated along the myeloid lineage implies that this expression is likely to be in macrophages. To confirm this, splenic macrophages/monocytes were purified using cell sorting

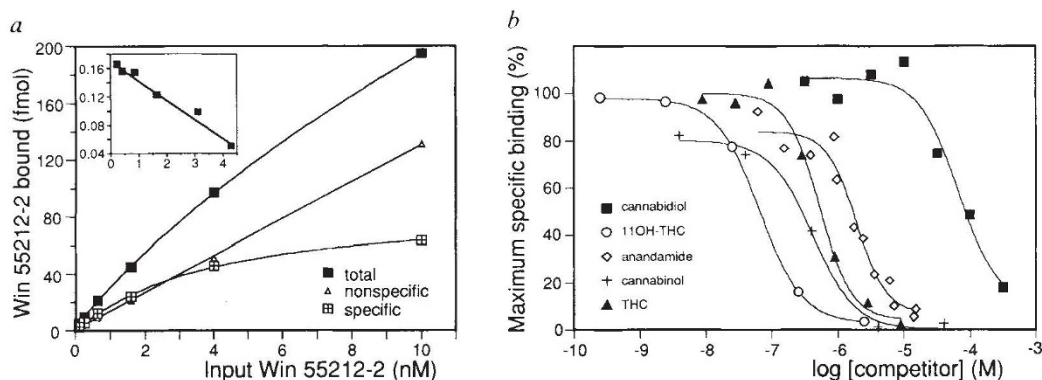


FIG. 2 Binding of cannabinoids to the receptor encoded by hCX5.36. *a*, Binding of [3 H]Win 55212-2 to membranes from COS cells transfected with an expression plasmid SC36, that contains the hCX5.36 cDNA. The inset plot shows the specific binding presented as bound/free against [bound] ($\text{M} \times 10^{-10}$). *b*, Displacement by cold cannabinoids of [3 H]CP55,940, or of [3 H]Win 55212-2, bound to membranes from COS cells transfected with SC36. **METHODS.** Plasmid SC36 is hCX5.36 inserted into the vector CDM8³⁰. 72 h after transfection, cells were Dounce homogenized and the membranes pelleted from the post-nuclear supernatant at 90,000g for 20 min, washed and then resuspended in 50 mM Tris-HCl pH 7.4, 3 mM MgCl_2 , 1 mM EDTA and stored in liquid N_2 . Binding of [3 H]Win 55212-2 ($49.6 \text{ Ci mmol}^{-1}$; New England Nuclear) to membranes ($40 \mu\text{g}$ membrane protein per $150 \mu\text{l}$ reaction), was determined essentially as

described, except that siliconized 1.5 ml polypropylene tubes were used for the binding reactions and 5% ethanol, 5% Triton X-100 was used to solubilize the membrane pellets⁵. Nonspecific binding was measured in the presence of $10 \mu\text{M}$ Δ^9 -THC, and data points shown are means of duplicates (average duplicate's difference 4.3%). Displacement by cold cannabinoids (Sigma) was determined using 1.0 nM [3 H]CP55,940 (107 Ci mol^{-1} ; New England Nuclear), or for anandamide (provided by R. Mechoulam) and cannabidiol, 1.0 nM [3 H]Win55212-2, although similar results were obtained for all competitors with both hot ligands (not shown). The anandamide displacement curve comprises data from two separate experiments. All data points are means of duplicates and all experiments were repeated at least twice. Inhibition constants (K_i) in nM: 11-OH- Δ^9 -THC, 40 ± 1.5 ; Δ^9 -THC, 320 ± 80 ; cannabinal, 250 ± 80 ; cannabidiol, $38,000 \pm 18,000$.

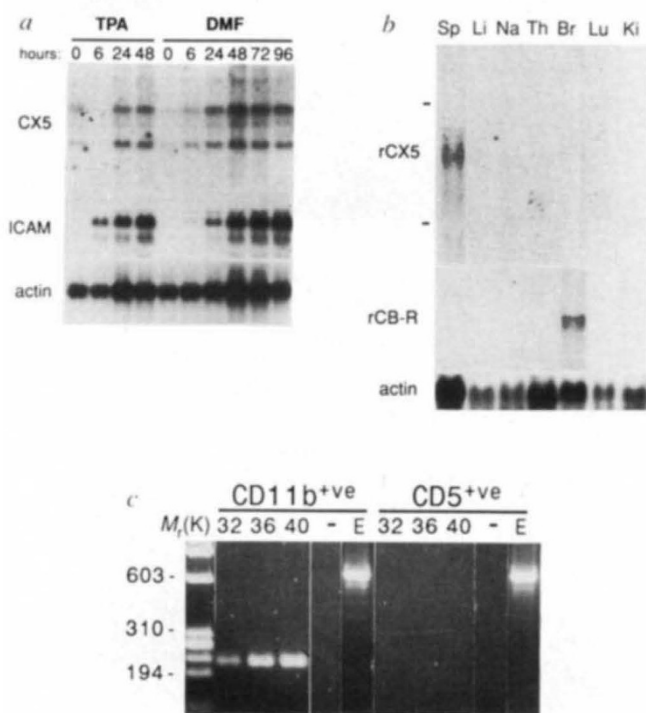


FIG. 3 Expression of CX5 transcripts in HL60 cells and in rat tissues. *a*, Northern blots of RNA from HL60 cells induced with either 20 ng ml^{-1} TPA or with 0.7% DMF, probed with either hCX5, or with ICAM-1 to follow induction. The hCX5 blot was then reprobated for γ -actin. *b*, Northern blot of RNA from various rat tissues probed with a rat homologue of CX5 (SP, spleen; Li, liver; Na, nasal epithelium; Th, thymus; Br, brain; Lu, lung; Ki, kidney). The blot was then reprobated with the rat cannabinoid receptor (rCB-R) and then with actin. *c*, PCR analysis of rCX5 expression in sorted rat spleenocytes. cDNA from rat spleenocytes sorted using antibodies against CD11b for macrophages/monocytes, or CD5 for T-

cells, was amplified with primers specific for rCX5, with products being removed after the indicated number of cycles (32,36,40). To demonstrate that the rCX5 signal derives from mRNA, cDNA reactions without added reverse transcriptase were amplified for 40 cycles (—) and, as a positive control, cDNAs were amplified with primers specific for elongation factor 1α (E).

METHODS. Total RNA was isolated from HL60 cells by lysis in guanidinium/LiCl, and $7.5 \mu\text{g}$ per sample was separated on a 1.2% agarose/4% formaldehyde gel, transferred to nylon (Hybond-N, Amersham) and ultraviolet cross-linked. Parallel blots were probed at 42°C in $5 \times \text{SSPE}/50\%$ formamide/ $100 \mu\text{g/ml}$ salmon sperm DNA/ $5 \times$ Denhardt's/ 0.1% SDS with either CX5 or ICAM-1²⁹ labelled with ^{32}P by random-priming (Pharmacia). After washing with $1 \times \text{SSC}$ at 42°C , the blot was exposed to Kodak XAR with an intensifying screen, ICAM, 8 days; hCX5, 10 days. The blots were then stripped according to the manufacturer's instructions and reprobated with human γ -actin, exposure 6 h. The fall in expression at the 6 h time point in TPA was reproducible. A rat homologue of CX5 was cloned from genomic DNA by PCR using primers. GGGCTCGAGGTNRAYTTYCAYGNTT and GAGGGATCCATNSWRCARAANGCRAA that encode sequences in hCX5 which are also found in the cannabinoid receptor but not in other G-protein-coupled receptors (VNFHV (91–96) and FAFCSM (279–284)). Cloning of PCR products of 600–650 bp produced primarily the rat cannabinoid receptor or a sequence with 88% homology to hCX5, which was termed rCX5 (S.M. unpublished observations). Total RNA extracted by guanidinium lysis from various rat tissues ($5\text{--}10 \mu\text{g}$ per lane) was blotted and probed as before with rCX5, rat cannabinoid receptor and γ -actin and then exposed using a phospho-imager (Molecular Dynamics) for rCX5 (9 h), CB-R (4 h) or XAR film (12 h) for actin. For PCR analysis, rat spleenocytes were separated by FACS using MRC OX-42 (CD11b)³¹ or MRC OX-19 (CD5)³² (Serotec). Cytoplasmic RNA prepared from 3×10^5 cells by detergent lysis, ($20 \mu\text{g}$ glycogen added as carrier) was treated with ribonuclease-free DNase (0.5 unit for 10 min; RQ1, Promega), phenol extracted, ethanol precipitated, resuspended in reverse transcriptase buffer with random primers, divided in two and MMLV reverse transcriptase was added to one set (GIBCO). After 60 min at 37°C , PCR amplification was done with the primers TTTCAGGTGTGGACTCC and TAGGTAGGAGATCAAGCG (rCX5, 214 bp product) or GAAATGCACCATGAAGCT and TTACGATGCATTGTTATC (EF-1 α , 645 bp product from spliced transcript³³) using 94°C , 1 min 54°C , 1 min, 72°C 1 min with the manufacturer's buffer (Promega).

and the expression of CX5 examined using PCR. Expression was detected in the macrophage/monocyte population but not in the CD5-positive population used as a control.

The marginal zone is the site through which blood-borne cells and antigens enter the spleen, and the marginal zone macrophages comprise a distinct population of highly phagocytic cells thought to play a role in both digesting and processing bacterial

antigens and in directing lymphocyte recirculation^{19, 21}. We are currently preparing antibodies specific for CX5 to investigate its cellular distribution in more detail, but the *in situ* distribution suggests that its expression is concentrated in these marginal zone macrophages. The *in vivo* function of CX5 is presumably to transduce a signal through a G-protein in response to an endogenous ligand, although we do not yet have direct evidence

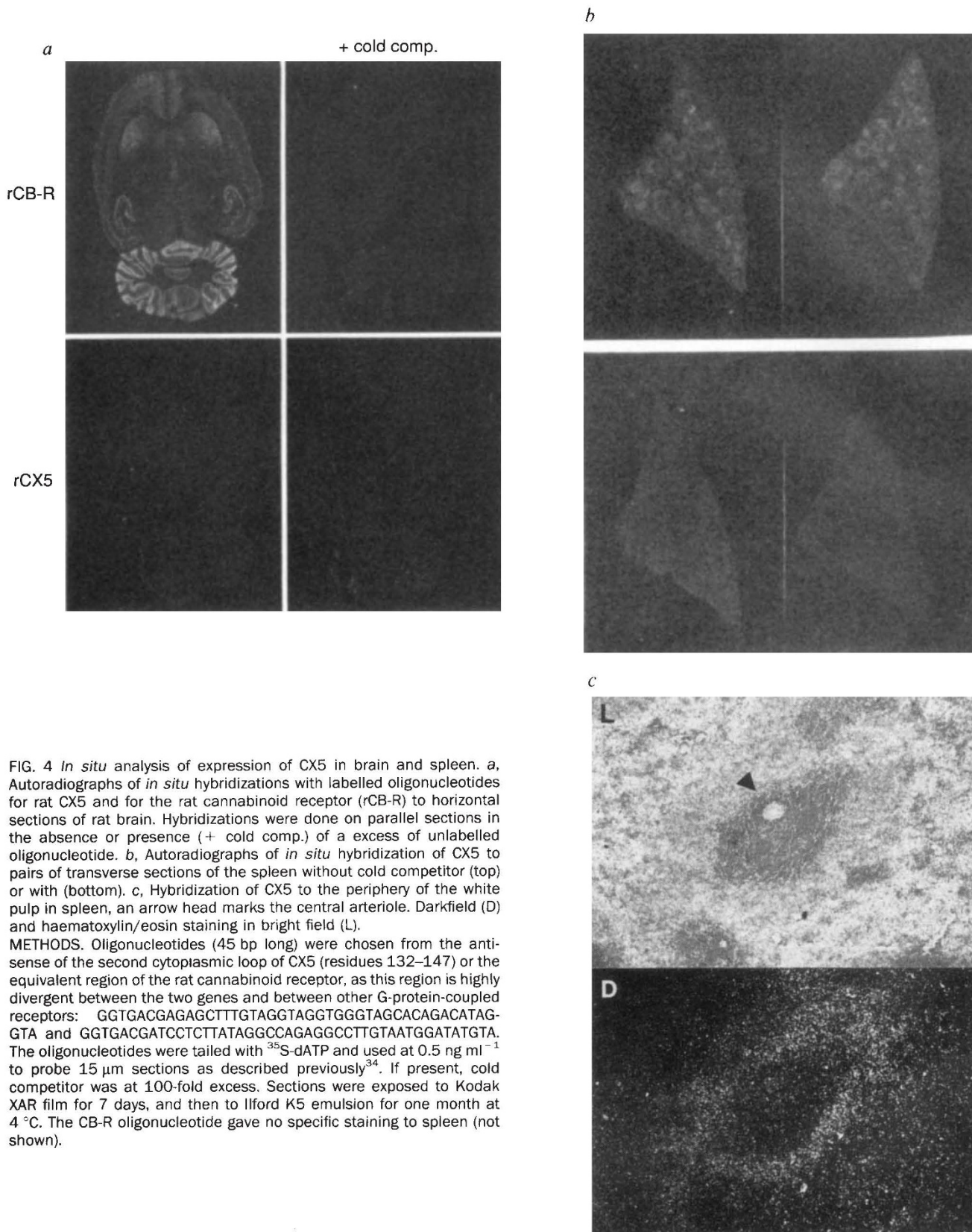


FIG. 4 *In situ* analysis of expression of CX5 in brain and spleen. a, Autoradiographs of *in situ* hybridizations with labelled oligonucleotides for rat CX5 and for the rat cannabinoid receptor (rCB-R) to horizontal sections of rat brain. Hybridizations were done on parallel sections in the absence or presence (+ cold comp.) of a excess of unlabelled oligonucleotide. b, Autoradiographs of *in situ* hybridization of CX5 to pairs of transverse sections of the spleen without cold competitor (top) or with (bottom). c, Hybridization of CX5 to the periphery of the white pulp in spleen, an arrow head marks the central arteriole. Darkfield (D) and haematoxylin/eosin staining in bright field (L).

METHODS. Oligonucleotides (45 bp long) were chosen from the anti-sense of the second cytoplasmic loop of CX5 (residues 132–147) or the equivalent region of the rat cannabinoid receptor, as this region is highly divergent between the two genes and between other G-protein-coupled receptors: GGTGACGAGAGCTTTGTAGGTAGGTGGGTAGCACAGACATAG-GTA and GGTGACGATCCTCTTATAGGCCAGAGGCCTTGTAAATGGATATGTA. The oligonucleotides were tailed with ³⁵S-dATP and used at 0.5 ng ml⁻¹ to probe 15 µm sections as described previously³⁴. If present, cold competitor was at 100-fold excess. Sections were exposed to Kodak XAR film for 7 days, and then to Ilford K5 emulsion for one month at 4 °C. The CB-R oligonucleotide gave no specific staining to spleen (not shown).

for such coupling. Analysis of other seven spanning receptors has suggested that basic, amphipathic helices at either end of the third cytoplasmic loop are involved in G-protein coupling and appropriate residues are found in these positions both in the CX5 sequence and in the brain receptor, which has been shown to be G-protein coupled^{6,22}.

There are many reports of cannabinoids exerting suppressive effects on various cells of the immune system, including macrophages²³⁻²⁵, although the significance of some of these observations has been questioned because of the high doses of drug used²⁶. But the location of the CX5 receptor, and its distinct structure from the brain receptor, strongly suggest that the endogenous ligand for these receptors will have an immuno-modulatory role in addition to its neuronal function. Anandamide has been recently identified as a candidate ligand for the cannabinoid receptor¹⁶ and this compound also binds to the CX5 receptor, although with an apparent affinity 30-fold less than that reported for the brain receptor. Anandamide is able to cross the blood brain barrier rapidly²⁷ but worthwhile speculation as to its function, and possible interactions between the neural and immunological systems, will require the identification of all the sources of this intriguing molecule. Furthermore, the question of further potential ligands from brain remains to be resolved¹⁶. Even so the existence of the CX5 receptor does have further implications. G-protein-coupled receptors are highly conserved throughout evolution¹², and yet the sequence of CX5 is considerably divergent from that of CB-R. Of the 162 residues in transmembrane sections of the human CB-R, three are different in rat CB-R, but 68 are different in human CX5. This suggests that the two receptors did not diverge recently and furthermore it suggests that it should be possible to identify receptor-specific cannabinoids. The fact that cannabinol appears to have a higher relative affinity for the CX5 receptor than for the brain receptor, may provide the basis for identifying such a ligand for the CX5 receptor. We suggest that in future the two receptors be distinguished by calling the brain receptor CB1 and the CX5 receptor CB2. It has been proposed that the peripheral effects of cannabinoids are either indirect effects of central actions, or reflect interactions with non-receptor proteins such as lipoxygenases^{1,8}. It is clearly possible that some of these peripheral effects are in fact mediated through the CB2 receptor and it will be interesting to determine the activities of any cannabinoids specific for this receptor. □

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Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinaemia

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THE microsomal triglyceride transfer protein (MTP), which catalyses the transport of triglyceride, cholesteryl ester and phospholipid between phospholipid surfaces, is a heterodimer composed of the multifunctional protein, protein disulphide isomerase, and a unique large subunit with an apparent M_r of 88K (refs 1-3). It is isolated as a soluble protein from the lumen of the microsomal fraction of liver and intestine⁴. The large subunit of MTP was not detectable in four unrelated subjects with abetalipoproteinaemia⁵, a rare autosomal recessive disease characterized by a defect in the assembly or secretion of plasma lipoproteins that contain apolipoprotein B (ref. 6). We report here the isolation and sequencing of complementary DNA encoding the large subunit of MTP. A comparison of this sequence to corresponding genomic sequences from two abetalipoproteinaemic subjects revealed a homozygous frameshift mutation in one subject and a homozygous nonsense mutation in the other. The results indicate that a defect in the gene for the large subunit of MTP is the proximal cause of abetalipoproteinaemia in these two subjects, and that MTP is required for the secretion of plasma lipoproteins that contain apolipoprotein B.

Based on the sequence of 1 of 10 peptides isolated from the large subunit of MTP (Fig. 1), a 20-base, 32-fold degenerate oligonucleotide probe was designed and used to screen a λ gt10, bovine small intestine cDNA library. Overlapping bovine clones

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