

Negative feedback regulation of IgE synthesis by murine CD23

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IMMUNOGLOBULIN E is found in nanogram amounts in normal human and mouse serum. It is increased during parasitic infestations¹ and mediates allergy. CD23, the low-affinity receptor for IgE (FcεRII), has been proposed as an important regulator of IgE synthesis²⁻⁴. The type-II transmembrane lectin⁴ CD23 is expressed in the mouse on B cells and follicular dendritic cells. In humans there are two forms of CD23 which differ in their intracellular amino-terminal 6/7 amino acids⁴; expression of the A-form corresponds to that of murine CD23, whereas the B-form is also found on T and other haematopoietic cells⁴. CD23 has been implicated in cellular adhesion⁵, antigen presentation⁶, as a growth and differentiation factor for human B, T and plasma cells, and as a signal transduction molecule⁷ (reviewed in refs 3, 8). Here we disrupt the gene coding for murine CD23 (ref. 9) to clarify the role of CD23 *in vivo* and find that B- and T-cell development is normal in these CD23-deficient mice. Immune responses to the helminth *Nippostrongylus brasiliensis* are unaffected. In contrast, immuni-

zation with thymus-dependent antigens leads to increased and sustained specific IgE antibody titres compared with controls. Formation of germinal centres is normal. These results suggest that murine CD23 acts as a negative feedback component of IgE regulation.

Our targeting strategy was aimed at inactivating the transmembrane domain encoded by exon 3 of *Fcer2a*¹⁰. Exons 3 and 4 were replaced by a neomycin-resistance (*neo*^r) cassette in a C57BL/6-derived embryonic stem (ES) cell line¹¹ (Fig. 1a). Southern blot analysis showed a proper targeting event (Fig. 1b). The absence of messenger RNA for CD23 and membrane-bound protein was demonstrated by northern blot analysis (Fig. 1c) and flow cytometry (Fig. 1d), respectively.

The composition of the lymphoid compartments and the differentiation of lymphocytes were studied by multi-parameter flow cytometry. The number of B and T cells in peripheral lymphoid organs did not differ between mutant and wild-type mice. Maturation of B cells in the bone marrow was not affected using CD24, CD44, CD45, BP-1, IgD and IgM as differentiation markers. B-cell subpopulations, defined as B1 or B2 subsets, were unchanged. The T-cell compartment in the thymus and the periphery was unaffected, as judged by the expression of the surface markers CD3, CD44, CD24, CD25 and CD69 (data not shown). The absence of CD23 apparently does not impair B- and T-cell development. These results do not support proposals that CD23 is a growth factor for human B and T cells^{12,13}.

In CD23-deficient mice, serum levels of IgM, IgG3 and IgA were not affected. But IgG1 and IgG2b levels were twofold lower, whereas IgE levels were increased twofold as compared with controls. To measure the half-life of IgE, mice were injected with serum obtained from IgE-transgenic mice¹⁴. The rate of disappearance of IgE was identical in CD23-deficient and control mice (Fig. 2). These results indicate that CD23 is not essential for class switch or for plasma-cell differentiation¹⁵ and clearance of serum IgE.

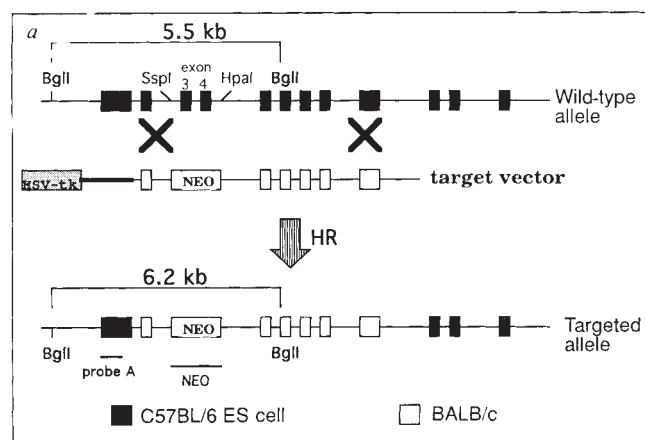
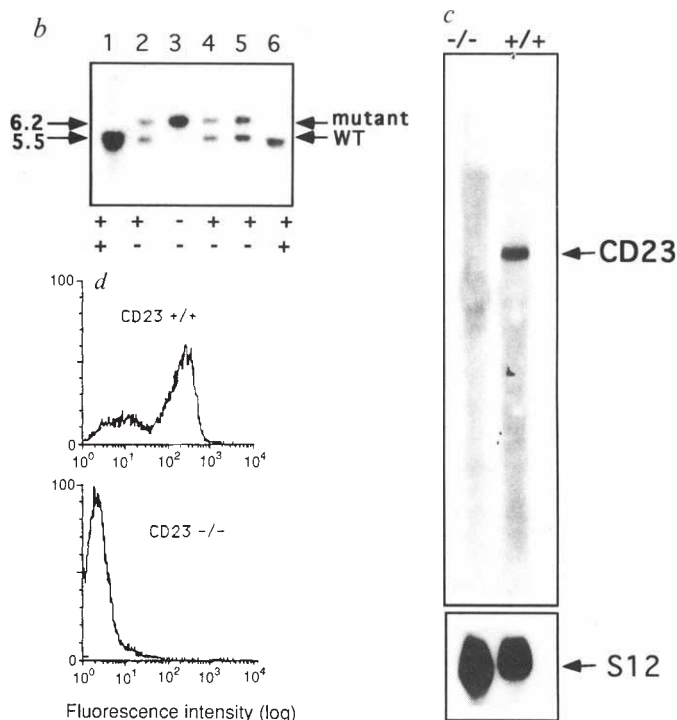
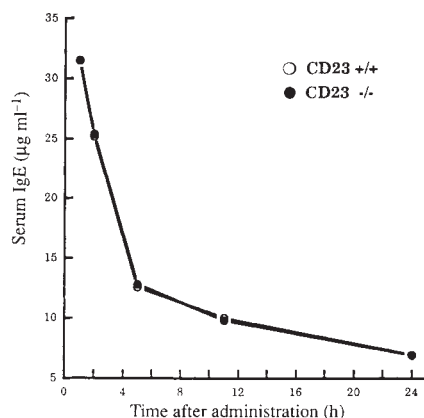


FIG. 1 Gene targeting of *Fcer2a*, the gene coding for murine CD23. a, Strategy for homologous recombination. A 408-base-pair (bp) *SspI*-*HpaI* fragment containing exons 3 and 4 was replaced by a *neo*^r cassette. The homologies on the short arm and the long arm are 1.1 kilobases (kb) and 3.4 kb, respectively. We positioned a herpes simplex virus thymidine kinase (HSV-tk) cassette at the 5' end of the vector. Isolation of clones and screening by PCR has been described²⁷. HR, homologous recombination. b, Southern blot analysis. Genomic DNA from neomycin-resistant ES cell lines or ES-cell-derived offspring was digested with *Bgl*II. Southern hybridization with probe A confirmed the disruption of the *Fcer2a* gene. The wild-type band is 5.5 kb and the targeted allele 6.2 kb. Lanes 1-4: tail DNA from different progeny (+/+), wild-type (WT); +/-, heterozygous mutant; and -/-, homozygous mutant; lane 5: *Fcer2a*-targeted ES-cell clone ISA 22; lane 6: C57BL/6 ES cell line (not manipulated). c, Northern blot of RNA of homozygous mutant (-/-) and wild-type (+/+) mice. 15 µg total splenic RNA was loaded per lane. Top, the blot was hybridized with a 600-bp fragment that covered coding



sequences from the exons 8 to 12; arrow, 2.2 kb. Bottom, same blot re-probed for the ribosomal protein S12 (ref. 28) to verify the amount of RNA loaded. d, Flow-cytometric analysis of spleen cells. B cells were labelled with antibodies B3B4 (ref. 29; anti-CD23, biotinylated and visualized with streptavidin-phycoerythrin) and 6B2 (anti-CD45 (B220)), a pan B-cell marker, labelled with fluorescein isothiocyanate (FITC). Histograms of B220⁺ cells are shown.

FIG. 2 Clearance of IgE in CD23-deficient and wild-type mice. Mice (2 per group) were injected intravenously with 400 μ l serum of IgE-transgenic mice¹⁴ containing 200 μ g ml⁻¹ IgE. At the indicated times after injection, total serum IgE was measured by enzyme-linked immunosorbent assay (ELISA)¹⁹.



We compared the immune response of CD23-deficient and wild-type mice using three protocols. First, mice were challenged with the thymus-independent antigen 2,4,6-trinitrophenyl-Ficoll (TNP-Ficoll). CD23^{-/-} mice mounted a twofold higher IgM response (not shown). Next, antibody titres were measured following immunization with the thymus-dependent antigen 2,4-dinitrophenyl-ovalbumin (DNP-OVA). Anti-DNP titres for IgM, IgG2b and IgG3 were comparable. In contrast, specific IgG1 was twice as high, and specific IgE 6–12 times as high, in CD23^{-/-} mice (Fig. 3a, b). Total IgE was also raised (Fig. 3c), and in addition, the decrease of IgE in CD23^{-/-} mice was slower (Fig. 3b, c). Similar results were obtained in CD23-deficient mice after immunization with another haptenated antigen, 4-hydroxy-3-nitrophenyl-OVA (NP-OVA; results not shown), and the non-haptenated antigen keyhole limpet haemocyanin (KLH) (Fig. 3d, e).

The formation of germinal centres¹⁵ was investigated in mice immunized with DNP-OVA. Development of germinal centres in CD23^{-/-} mice was not affected (Fig. 4). Interestingly, although

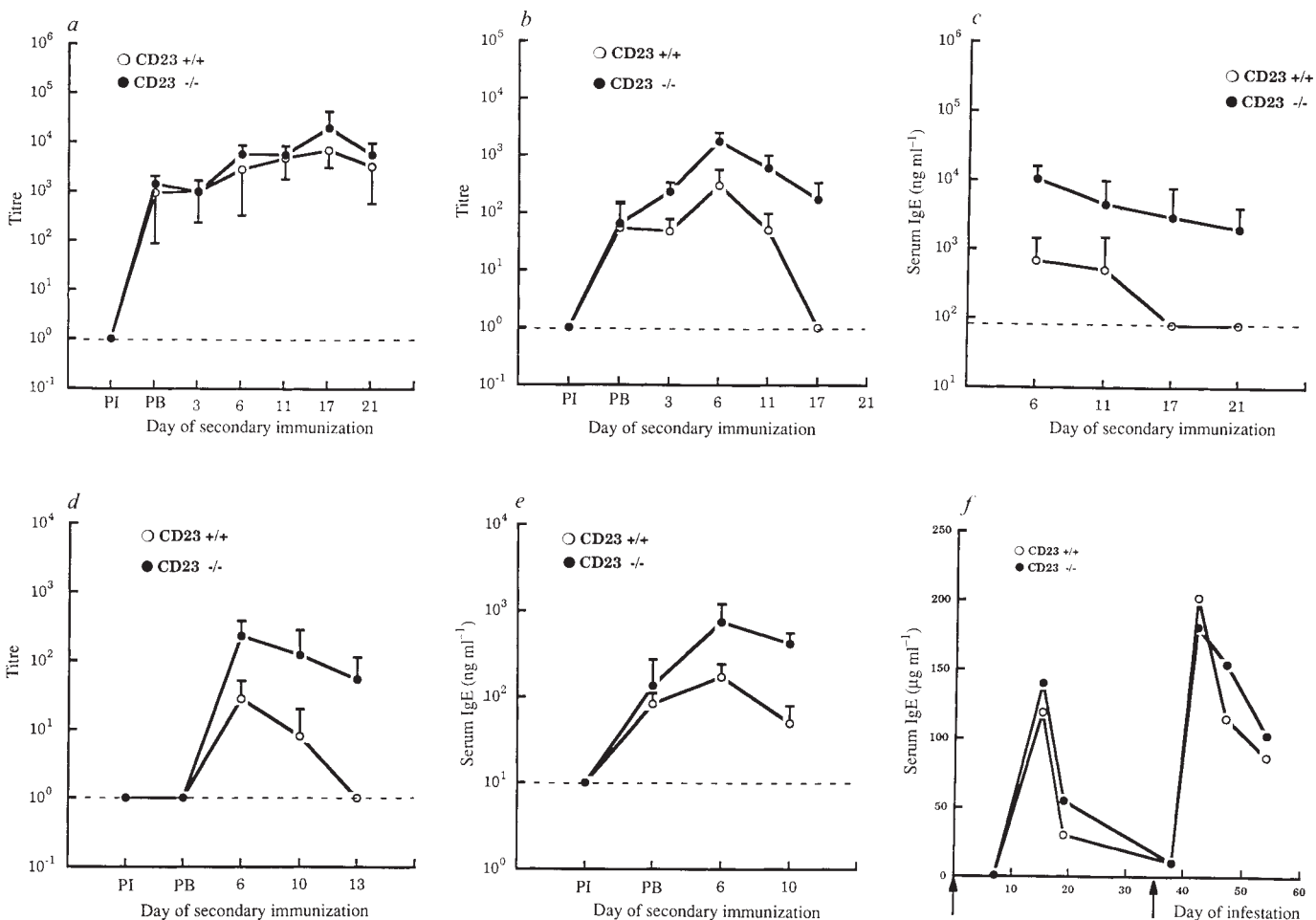


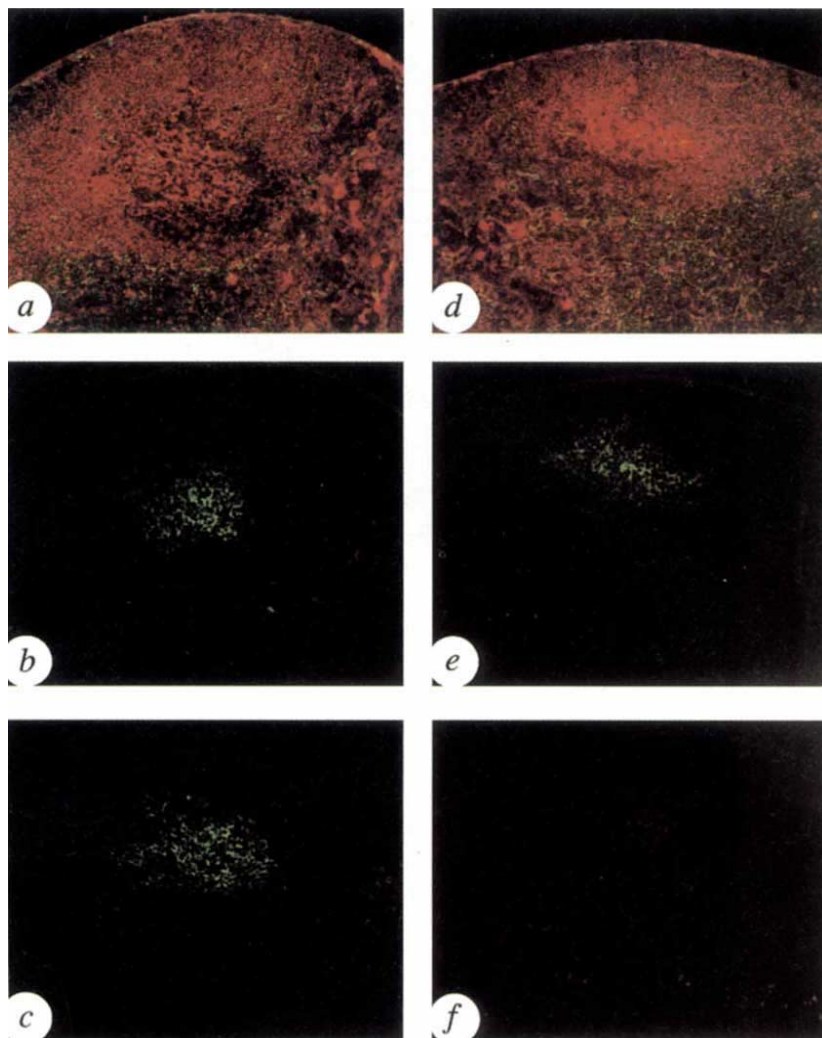
FIG. 3 Immunization experiments and serum antibody levels. a, DNP-specific IgG1; b, DNP-specific IgE; and c, total IgE, in DNP-OVA-immunized mice; d, KLH-specific IgE; e, total IgE in KLH-immunized mice; and f, total IgE in *N. brasiliensis*(Nb)-infested mice. a–e, Mice were immunized subcutaneously (s.c.) with 20 μ g DNP-OVA or 20 μ g KLH (Calbiochem) precipitated in alum with *Bacillus pertussis*. Three weeks later, they were immunized intraperitoneally (i.p.). Serum was taken at different times before and after booster immunizations. Results are expressed as the serum dilution where half-maximum absorbance values were obtained (a, b, d). Serum IgE levels were calculated from serially diluted serum samples (c, e, f). Values from individual mice were used to determine arithmetic means ($\pm$ s.d.). PI, pre-immune; PB, pre-booster. Dashed line represents the lower detection limit. The detection of KLH-specific IgE antibodies was facilitated by depletion of

IgG antibodies: serum was diluted to 200 μ l with PBS buffer and added to 50 μ l packed protein G-Sepharose beads (Pharmacia). After overnight depletion at 4 °C, the supernatant was used for ELISA. Murine IgE did not bind to protein G (manuscript in preparation). f, Mice were immunized s.c. with 700 *N. brasiliensis* larvae stage 3 (Nb L3) at day 0 and again after 35 days (arrows). Serum was taken before and at different times after primary and secondary infestations. Results are given as arithmetic means of serum IgE levels of individual mice.

METHODS. For immunization experiments, 10 eight-week-old animals (DNP-OVA and Nb) or 6 six-week-old animals (KLH) were used per group. Serum levels were determined by ELISA¹⁹. Specific antibodies were determined by ELISA using DNP-bovine serum albumin or KLH as antigen¹⁹.

FIG. 4 Immunohistological evaluation of germinal-centre development, CD23 expression and IgE localization. Serial cryostat sections were prepared from lymph nodes of DNP-OVA-immune mice nine days post-secondary immunization. *a, b* And *c* are from wild-type mice, and *d, e* and *f* are from CD23-deficient mice. *a* And *d* show labelling of IgM-positive B cells in red and Thy1-positive T cells in green. Note the strong red immune-complex labelling of the FDC network positioned adjacent to the IgM-negative area of the germinal centre dark zone. *b* and *e* demonstrate the ability of the FDC to trap immune complexes containing IgE; *c* shows the level of CD23 expression in wild-type mice, and *f* confirms the absence of this molecule in the deficient mouse.

METHODS. 10- μ m-thick frozen sections were prepared from lymph nodes taken 9 days post-secondary challenge with DNP-OVA in alum with *B. pertussis*. The expression of cell-surface molecules was visualized using sheep anti-mouse IgM-Texas Red (Southern Biotechnology), and T24 (anti-Thy1), B3B4 (ref. 29; anti-CD23) or 1-5 (anti-IgE; gift from G. Le Gros) with a mouse anti-rat IgG (specific for heavy and light chain) F(ab')₂ conjugated to FITC reagent (Jackson Immuno Research).



follicular dendritic cells (FDCs) of deficient mice did not express CD23 (Fig. 4*f*), IgE was detected on the surface of these cells (Fig. 4*e*). This indicates that IgE can be bound to FDCs either directly or complexed with other molecules such as complement components and their receptors¹⁶. Our results show that CD23 is not essential in the initiation and maintenance of an immune response. The proposed role of CD23 in antigen presentation^{6,17} is normally of minor importance *in vivo*.

The final immunization protocol involved infestation with the helminth *N. brasiliensis*, which induces strong IgE production¹⁸. Antibody production was similar in both types of mice with respect to total IgM, IgG1, IgG2b, IgG3 and IgA (not shown). CD23^{-/-} mice did not produce significantly more serum IgE than wild-type mice (Fig. 3*f*). Therefore, it seems that CD23 is not involved in primary or secondary parasite-induced antibody responses.

We propose that the function of CD23 lies in a negative feedback control of IgE. It is noteworthy that the interleukin IL-4 is not only essential for class switch to IgE¹⁹ but is also a strong inducer of CD23 expression²⁰. In an IgE-transgenic mouse model it was shown that binding of monomeric IgE to CD23 and subsequent increased expression of CD23 are not sufficient to interfere with antigen-specific IgE responses¹⁴. We therefore postulate that binding of IgE-containing immune complexes to CD23 renders the B cell unresponsive to further stimulation or directly suppresses IgE synthesis. Further support for this model comes from the observations: (1) anti-CD23 antibodies or IgE-containing immune complexes suppress human B-cell proliferation²¹ and IgE production *in vitro*^{22,23}; and (2) anti-CD23 antibody

treatment of rats inhibits a specific IgE immune response². The latter observation was interpreted to favour a role for CD23 as an inducer of IgE synthesis. Alternatively, CD23 might convey a negative signal, in support of our hypothesis. The observation that CD23 does not regulate IgE synthesis in infestation with *N. brasiliensis* can be attributed to the dominant activation of Th-2 cells²⁴ and the strong T-cell-independent, nonspecific activation of B cells¹⁸ by the helminth that overrides the regulatory function of CD23.

In conclusion, murine CD23 is a negative controlling element in IgE production. Its function is reminiscent of that of Fc γ R, which also turns off B cells after binding specific immune complexes^{25,26}. A dysfunction of CD23 may play a part in the aetiology of IgE-mediated diseases such as atopy, allergy and asthma. □

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- Hagan, P., Blumenthal, U. J., Dunn, D., Simpson, A. J. G. & Wilkins, H. A. *Nature* **349**, 243–245 (1991).
- Flores-Romo, L. et al. *Science* **261**, 1038–1041 (1993).
- Conrad, D. H. A. *Rev. Immun.* **8**, 623–645 (1990).
- Sutton, B. J. & Gould, H. J. *Nature* **366**, 421–428 (1993).
- Aubry, J.-P., Pochon, S., Graber, P., Jansen, K. U. & Bonnefoy, J.-Y. *Nature* **358**, 505–507 (1992).
- Kehry, M. R. & Yamashita, L. C. *Proc. natn. Acad. Sci. U.S.A.* **86**, 7556–7560 (1989).
- Kolb, J.-P. et al. *J. Immun.* **150**, 4798–4809 (1993).
- Gordon, J. (ed.) *Monogr. Allergy: CD23* **29** (Karger, Basel, 1991).
- Thomas, K. R. & Capecci, M. R. *Cell* **51**, 503–512 (1987).
- Richards, M. L., Katz, D. H. & Liu, F. T. *J. Immun.* **147**, 1067–1074 (1991).
- Ledermann, B. & Bürki, K. *Expl. Cell Res.* **197**, 254–258 (1991).
- Swendeman, S. & Thorley-Lawson, D. A. *EMBO J.* **6**, 1637–1642 (1987).
- Bertho, J.-M., Fourcade, C., Dalloul, A. H., Debré, P. & Mossalayi, M. D. *Eur. J. Immun.* **21**, 1073–1076 (1991).

14. Adamczewski, M., Köhler, G. & Lamers, M. C. *Eur. J. Immun.* **21**, 617–626 (1991).
15. Liu, Y.-J., Johnson, G. D., Gordon, J. & MacLennan, I. C. M. *Immun. Today* **13**, 17–21 (1992).
16. Maeda, K. et al. *J. Immun.* **148**, 2340–2347 (1992).
17. Heyman, B., Tianmin, L. & Gustavsson, S. *Eur. J. Immun.* **23**, 1739–1742 (1993).
18. Lebrun, P. & Spiegelberg, H. L. *J. Immun.* **139**, 1459–1465 (1987).
19. Kopf, M. et al. *Nature* **362**, 245–247 (1993).
20. Keegan, A. D., Snapper, C. M., Van Dusen, R., Paul, W. E. & Conrad, D. H. *J. Immun.* **142**, 3868–3874 (1989).
21. Luo, H., Hofstetter, H., Banchereau, J. & Delespesse, G. *J. Immun.* **146**, 2122–2129 (1991).
22. Sherr, E., Macy, E., Kimata, H., Gilly, M. & Saxon, A. *J. Immun.* **142**, 481–489 (1989).
23. Saxon, A., Kurbe-Leamer, M., Behle, K., Max, E. E. & Zhang, K. *J. Immun.* **147**, 4000–4006 (1991).
24. Street, N. E. et al. *J. Immun.* **144**, 1629–1639 (1990).
25. Campbell, K. A., Lees, A., Finkelman, F. D. & Conrad, D. H. *Eur. J. Immun.* **22**, 2107–2112 (1992).

26. Sinclair, N. R. *StC. Immun. Today* **4**, 35–36 (1983).
27. Nitschke, L., Kopf, M. & Lamers, M. C. *BioTechniques* **14**, 914–916 (1993).
28. Ayane, M. P., Neilsen, P. & Köhler, G. *Nucleic Acids Res.* **17**, 6722 (1990).
29. Rao, M., Lee, W. T. & Conrad, D. H. *J. Immun.* **138**, 1845–1851 (1987).

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A mammalian protein targeted by G1-arresting rapamycin–receptor complex

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THE structurally related natural products rapamycin and FK506 bind to the same intracellular receptor, FKBP12, yet the resulting

complexes interfere with distinct signalling pathways^{1,2}. FKBP12–rapamycin inhibits progression through the G1 phase of the cell cycle in osteosarcoma³, liver^{4,5} and T cells^{6,7} as well as in yeast⁸, and interferes with mitogenic signalling pathways that are involved in G1 progression^{9,10}, namely with activation of the protein p70^{S6k} (refs 5, 11–13) and cyclin-dependent kinases^{3,14–16}. Here we isolate a mammalian FKBP–rapamycin-associated protein (FRAP) whose binding to structural variants of rapamycin complexed to FKBP12 correlates with the ability of these ligands to inhibit cell-cycle progression. Peptide sequences from purified bovine FRAP were used to isolate a human cDNA clone that is highly related to the *DRR1/TOR1* and *DRR2/TOR2* gene products from *Saccharomyces cerevisiae*^{8,17,18}. Although it has not been previously demonstrated that either of the *DRR/TOR* gene products can bind the FKBP–rapamycin complex directly^{17,19}, these yeast genes have been genetically linked to a rapamycin-sensitive pathway and are thought to encode lipid kinases^{17–20}.

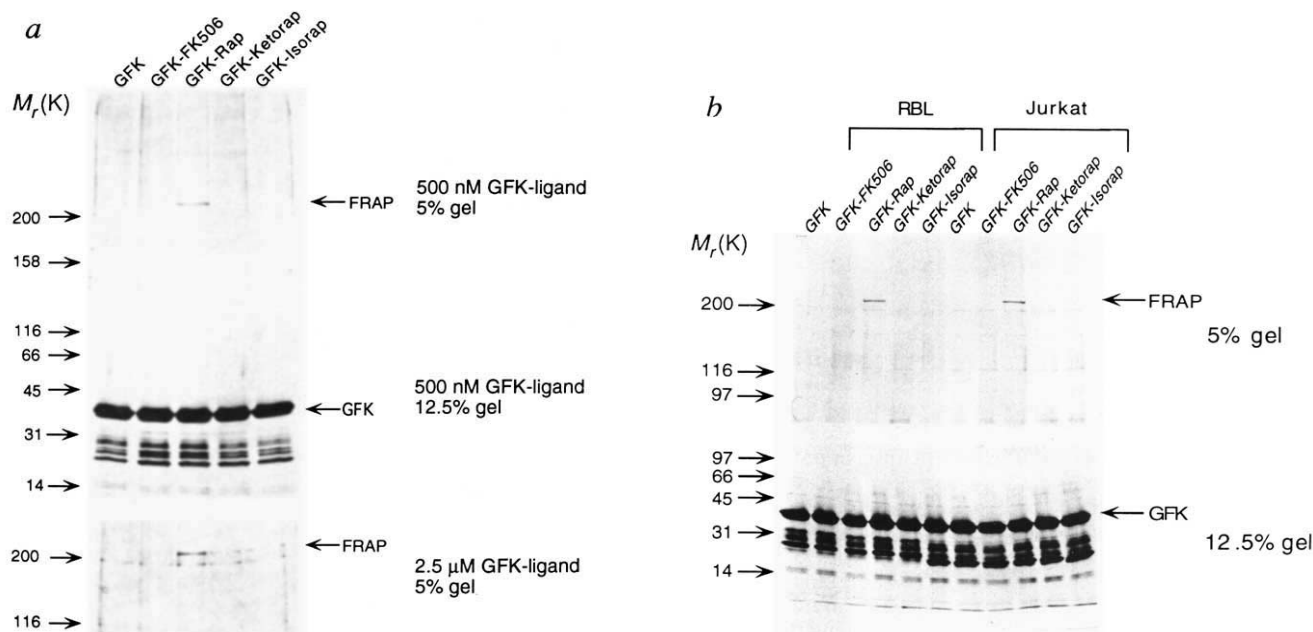


FIG. 1 Identification of FRAP protein in three mammalian cell lines. *a*, GFK alone or individual GFK–ligand complexes were added to MG-63 cell lysates (2×10^7 cells per condition) to a final concentration of either 500 nM or 2.5 μ M and the mixtures incubated for 10 min at 4 °C. Fusion protein complexes were recovered by glutathione-affinity chromatography, and the proteins detected by silver staining after 5% SDS-PAGE. Because of compression, FRAP is not resolved by 12.5% SDS-PAGE, so both 5% and 12.5% gels are shown. The amount of FRAP that was retained by affinity chromatography saturated at concentrations of GFK–Rap greater than 500 nM in these experiments and in others using concentrations of GFK–Rap ranging from 100 nM to 5 μ M (data not shown). *b*, GFK alone or individual GFK–ligand complexes were added to a final concentration of 500 nM to lysates prepared from either 2×10^8 Jurkat T lymphocytes or 10^8 rat basophilic leukaemia (RBL) cells per condition. Lysates were treated as in *a*. FKBP12, but not FKBP13 or FKBP25 (ref. 23) is able to mediate the actions of rapamycin in *S.*

cerevisiae. In addition, we found that YFK188 (ref. 24), an FKBP12 null strain, could be complemented with GFK (P. K. Martin, B. Gladstone, G. Weiss, D. T. Hung, S.L.S., in preparation). Thus the GST appendage of the fusion protein does not preclude binding of the biologically relevant target to the GFK–rapamycin complex in yeast. METHODS. MG-63, Jurkat and RBL cells were grown in media containing 10% FBS and lysed at 4 °C in PINT buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM EGTA, 25 mM NaF, 100 μ M Na_3VO_4 , 25 mM 2-glycerophosphate, 0.2 mM PMSF, 1 μ g ml^{-1} leupeptin, 1 μ g ml^{-1} pepstatin A and 2 mM DTT) containing 0.5% Triton X-100. Lysates were clarified by centrifugation at 25,000g, and the Triton X-100 in the supernatant was diluted to 0.33% by adding 0.5 vol PINT buffer. GFK prebound to stoichiometric quantities of FK506, keto- iso- or unmodified rapamycin was added to lysates as described. Each condition was then passed through a 250- μ l glutathione-Sepharose column, which was washed with PINT buffer containing 0.5 M NaCl and 0.3% Triton X-100.