the diverse pattern of switching we have observed may prime the immune system for the rapid control of future heterologous challenges. This would explain how protective immunity can be achieved after relatively few clinical attacks of malaria[12,22], despite the large repertoire of variant antigenic types[10,11].

Changes in antigenic[23] or cytoadherence phenotypes[24] have been associated with a family of high-molecular-weight proteins expressed at the red-cell surface. We have found that antigenic variation is also accompanied by size changes in these proteins (data not shown). Thus the biochemical, antigenic and adhesive phenotypes are all co-modulated by clonal antigenic variation and so these properties will not be stable characteristics of an isolate, but will be determined by the interaction between the parasite genotype and the host environment. The same parasite infecting different individuals may therefore give rise to a variety of phenotypes depending on the selective pressures exerted by the host. These pressures could include constitutive or induced levels of endothelial or red-cell surface receptor expression and the speed and effectiveness of the variant specific antibody response. Furthermore, in view of the apparent linkage between antigenic and adhesive phenotypes (Figs 2 and 3) and the large antigenic repertoire[10,11], additional host receptors may exist. If severe disease is associated with specific adhesive phenotypes, such as rosetting, auto-agglutination or ICAM-1 binding, characterizing the antigenic determinants of these pathogenic subsets of the total repertoire of variant antigenic types may form the basis of a realistic strategy to control severe disease.

Note added in proof: Data linking antigenic and cytoadherence phenotypes of *P. falciparum* IT derived clones has also been obtained by Biggs *et al.*[30]. □

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FK-506- and CsA-sensitive activation of the interleukin-2 promoter by calcineurin

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ANTIGEN recognition by the T-cell receptor (TCR) initiates events including lymphokine gene transcription[1], particularly interleukin-2, that lead to T-cell activation. The immunosuppressive drugs, cyclosporin A (CsA) and FK-506, prevent T-cell proliferation by inhibiting a Ca^{2+}-dependent event required for induction of interleukin-2 transcription[2]. Complexes of FK-506 or CsA and their respective intracellular binding proteins inhibit the calmodulin-dependent protein phosphatase, calcineurin, *in vitro*[3]. The pharmacological relevance of this observation to immunosuppression or drug toxicity is undetermined. Calcineurin, although present in lymphocytes[4], has not been implicated in TCR-mediated activation of lymphokine genes or in transcriptional regulation in general. Here we report that transfection of a calcineurin catalytic subunit increases the 50% inhibitory concentration (IC_{50}) of the immunosuppressants FK-506 and CsA, and that a mutant subunit acts in synergy with phorbol ester alone to activate the interleukin-2 promoter in a drug-sensitive manner. These results implicate calcineurin as a component of the TCR signal transduction pathway by demonstrating its role in the drug-sensitive activation of the interleukin-2 promoter.

To investigate calcineurin's role in activation of the interleukin-2 (IL-2) promoter, we cotransfected a plasmid containing the human IL-2 promoter linked to a reporter gene along with an expression plasmid encoding either a wild type or a deletion mutant (ΔCaM-AI) of a murine calcineurin catalytic subunit (Fig. 1*a*) into the human T-cell line, Jurkat. ΔCaM-AI was designed to mimic proteolysed forms of the phosphatase known to have Ca^{2+}-independent, constitutive phosphatase activity *in vitro*[5,6]. Neither cotransfection of wild type nor ΔCaM-AI activated the IL-2 promoter either alone or in the presence of ionomycin (Fig. 1*b*). Cotransfection of wild type hyperactivated the IL-2 promoter roughly twofold in the presence of phorbol myristyl acetate (PMA; 12-*O*-tetradecanoylphorbol-13-acetate) and ionomycin (Fig. 1*c*), but had no effect with PMA alone (Fig. 1*b*). Thus, the combination of PMA and ionomycin is more potent in the presence of transfected calcineurin than in its absence (Fig. 1*c*), suggesting that calcineurin phosphatase activity limits the level of transcription from the IL-2 promoter. As expected for a constitutively activated component of an essential Ca^{2+}-dependent signalling pathway, ΔCaM-AI acted in synergy with PMA, bypassing the Ca^{2+} requirement and inducing >150-fold activation of the IL-2 promoter (Fig. 1*b*). Cotransfection of ΔCaM-AI also increased promoter activity in the presence of both mitogens 3.5-fold (Fig. 1*c*). The dependence on ionomycin for maximal promoter activity may reflect activation of endogenous calcineurin and/or activation of Ca^{2+}-dependent, calcineurin-independent pathways.

To demonstrate that the observed results were directly attributable to IL-2 promoter activation, we investigated ΔCaM-AI's effect on a reporter plasmid in which the cytomegalovirus immediate-early (CMV-IE) promoter directs chloramphenicol

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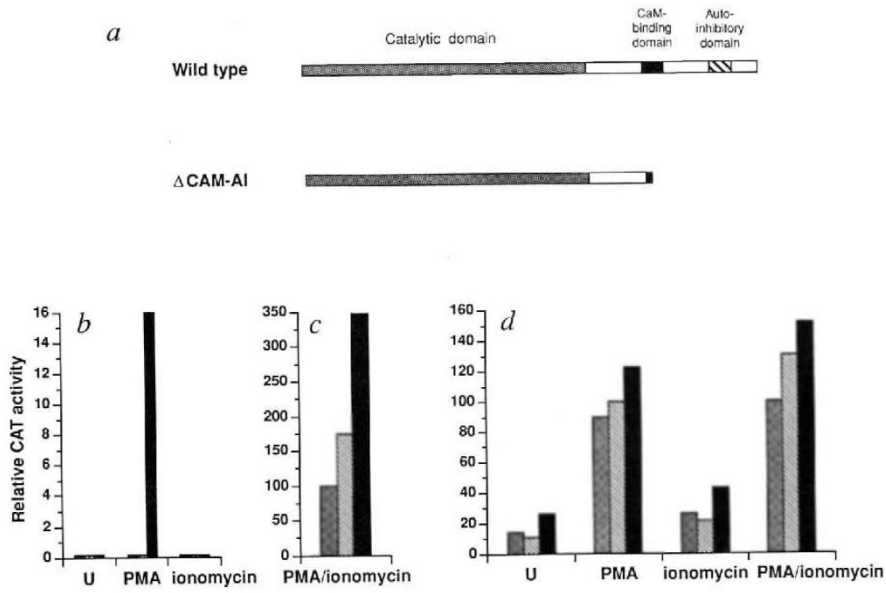

FIG. 1 A deletion mutant of murine calcineurin acts in synergy with PMA in FK-506-sensitive activation of the IL-2 promoter. *a*, Schematic representation of wild-type calcineurin catalytic subunit and the deletion mutant, ΔCaM-AI. The putative autoinhibitory[13] and CaM-binding domains[14] are indicated. Wild type was constructed by inserting CNα4[15], a complementary DNA clone encoding amino acids 1 to 521, into the *Eco*RI site of the eukaryotic expression vector pcDL-SRα296[16]. The deletion mutant was constructed by polymerase chain reaction (PCR) amplification of a CNα-4 template and insertion of a fragment encoding amino acids 1 to 398 into the *Sma*I site of pUC18[17]. Consequently, the 5′ and 3′ untranslated sequences present in CNα4 were also deleted. The clone was subsequently inserted into the *Eco*RI site of pcDL-SRα296. The plasmids are designated pSRα-calcineurin and pSRα-ΔCaM-AI, respectively. The wild type construct encodes a murine catalytic subunit 99% identical to the corresponding human subunit[15]. ΔCaM-AI lacks functional CaM-binding[14] and autoinhibitory[13] domains. This truncation was designed to mimic proteolysed forms of the phosphatase known to have Ca^{2+}-independent, constitutive phosphatase activity *in vitro*[5,6]. *b*, ΔCaM-AI transactivates the IL-2 promoter in synergy with PMA. The reporter, p(An)IL2.CAT, contains the human IL-2 promoter (base pairs −448 to +43) directing transcription of the CAT gene and includes an SV40 polyadenylation site between vector sequences and −448 of the IL-2 promoter to prevent vector-dependent transcription from contributing to the observed CAT activity. The p(An)IL2.CAT was cotransfected into Jurkat cells together with the effectors, pcDL-SRα296 (vector, ▩), pSRα-calcineurin (▧), or pSRα-ΔCaM-AI (■) and the transfection efficiency control, pCMV.Gal which contains the CMV-IE promoter directing transcription of the bacterial β-galactosidase (β-gal) gene. The transfected cells were activated as indicated 16 h after electroporation[7], incubated for another 16 h, collected and assayed. The data shown are an average of three or four independent experiments. *c*, Calcineurin hyperactivates the IL-2 promoter with PMA and ionomycin. Transfections were done as described above. *d*, ΔCAM-AI has no significant effect on the PMA-dependent stimulation of the CMV-IE promoter. The reporter, pCMV.CAT, contains the CMV-IE promoter (base pairs −760 to +4) directing transcription of the CAT gene. Transfections were done as described above. The data shown are an average of two independent experiments.

METHODS. Jurkat cells were passaged as described[7]. Cells were grown to a density of 4–6 × 10^5 ml^{-1}. Transfection was by electroporation (10^7 cells in 300 μl RPMI medium, 10% FCS, room temperature, 960 μF, 250 V, 0.4 cm cuvette, BioRad Gene Pulser) using 2 μg reporter plasmid, 15 μg of effector plasmid and 0.5–2 μg of the transfection efficiency control pCMV.Gal. Before activation or drug treatment each culture of transfected cells was divided into the appropriate number of aliquots. Cells were collected 12–16 h after treatment, lysed, and assayed for CAT[18] and β-gal[19] activities. The β-gal activity in PMA-treated cells was unaffected by the presence of the transfected calcineurin as demonstrated by addition of FK-506 to PMA-treated cells (data not shown). Relative CAT activity was adjusted for transfection efficiency as dictated by β-gal activity. For each promoter, the CAT activity induced by the indicated treatment is shown relative to the PMA/ionomycin-induced CAT activity of the vector control, which was set to 100.

acetyltransferase (CAT) gene transcription. This promoter has a measurable basal transcription activity and is stimulated by PMA in an FK-506-resistant manner[7]. ΔCaM-AI increases the basal activity of the CMV-IE promoter slightly (<twofold), but has virtually no effect on the PMA-stimulated activity (Fig. 1*d*). Thus, the effect of ΔCaM-AI is not due to stabilization of CAT message or activation of CAT enzyme.

If calcineurin is the FK-506-sensitive component in TCR signal transduction, then FK-506 should inhibit PMA/ΔCaM-AI-mediated activation of the IL-2 promoter. In fact, when FK-506 was added (to 10 ng ml^{-1}) immediately before PMA, activation of the IL-2 promoter was completely inhibited (Fig. 2*a*). Rapamycin, a macrolide that is structurally related to FK-506 but that does not inhibit calcineurin *in vitro*[3], had no effect at 100 ng ml^{-1} (Fig. 2*a*). Neither compound affected PMA-dependent activation of the CMV-IE promoter (Fig. 2*b*). Inhibition of ΔCaM-AI by FK-506 in the absence of ionomycin suggests that deletion of the autoinhibitory and calmodulin (CaM)-binding domains greatly reduces the Ca^{2+} requirement for binding of calcineurin to FK-506/FKBP[3]. Experiments in which trypsinized calcineurin is inhibited by CsA/cyclophilin A in the presence of 1 mM EGTA support this conclusion[8].

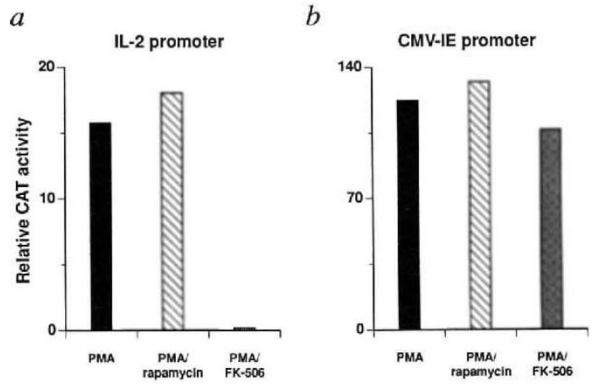

FIG. 2 FK-506 inhibits ΔCaM-AI-dependent activation of transcription. The plasmids p(An)IL2.CAT (*a*) and pCMV.CAT (*b*) were cotransfected with pSRα-ΔCaM-AI and the transfected cells were manipulated as described in Fig. 1. FK-506 (10 ng ml^{-1}, ▩) or rapamycin (100 ng ml^{-1}, ▧) was added immediately before addition of PMA (■) as indicated. Relative CAT activity is calculated as described in Fig. 1. The results presented in (*a*) are the average of four independent experiments and those in (*b*) of two independent experiments.

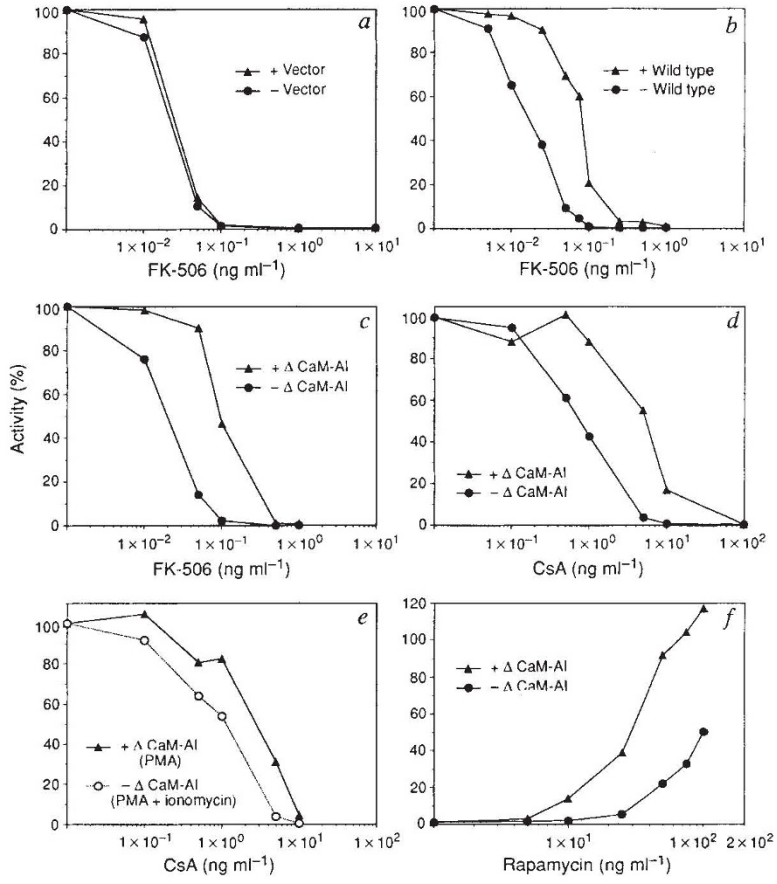

FIG. 3 Expression of the ΔCaM-AI mutant increases the IC_{50} of FK-506 and CsA and decreases the ED_{50} of rapamycin required to antagonize FK-506. All drug titrations were done with cell cultures that had been stimulated with the combination of PMA and ionomycin except as indicated. CAT (▲) and β-gal (●) activities are plotted as per cent of activity in lysates of transfected cells that were not treated with any immunosuppressive drug. *a*, Effect of vector on IC_{50} of FK-506. *b*, Effect of wild type on IC_{50} of FK-506. *c*, Effect of ΔCaM-AI on IC_{50} of FK-506. *d*, Effect of ΔCaM-AI on IC_{50} of CsA. *e*, Effect of ΔCaM-AI on IC_{50} of CsA in the absence of ionomycin. The β-gal titration (○) was determined from a parallel culture treated with the combination: PMA and ionomycin. *f*, Effect of ΔCaM-AI on ED_{50} of rapamycin.
METHODS. In each experiment Jurkat cells were cotransfected (as in Fig. 1) with p(An)IL2.CAT and either pcDL-SRα296 (*a*), pSRα-calcineurin (*b*), or pSRα-ΔCaM-AI (*c*–*f*), except the concentration of the effector plasmid was 30 μg per transfection and the pCMV.Gal was omitted. As an internal control for efficacy of the immunosuppressive drugs, Jurkat cells were transfected independently with 30 μg of a plasmid containing a derivative of the human IL-2 promoter directing transcription of the β-gal gene (pIL-2.Gal) and mixed with the CAT/effector cotransfected cells. After 24 h the cells were divided into aliquots and activated with PMA and ionomycin in the presence of the indicated concentrations of FK-506 (*a*–*c*), CsA (*d* and *e*), or 0.5 ng ml^{-1} FK-506 and the indicated concentrations of rapamycin (*f*). The cells were collected after 16 h and assayed as in Fig. 1.

Having postulated that hyperactivation of the IL-2 promoter by calcineurin expression plasmids is due to an increase in calcineurin concentration, we would predict that the IC_{50} of FK-506 should increase after transfection. The expression vector alone had no effect (Fig. 3*a*), but the IC_{50} of FK-506 increased 4–4.5-fold in the presence of both wild type and ΔCaM-AI. In representative experiments, the IC_{50} increased from 0.02 ng ml^{-1} to 0.08 ng ml^{-1} for wild type (Fig. 3*b*) and to 0.09 ng ml^{-1} for ΔCaM-AI (Fig. 3*c*). Similarly, ΔCaM-AI increases the IC_{50} of CsA over six-fold, from 0.8 ng ml^{-1} to 5 ng ml^{-1} in the depicted titration (Fig. 3*d*). The wild-type calcineurin cannot bind FK-506/FKBP in the absence of Ca^{2+} *in vitro*[3]; therefore, if the concentration of endogeneous calcineurin is significant relative to that of the transfected calcineurin, the IC_{50} of CsA or FK-506 with ΔCaM-AI and PMA should be lower than that with ΔCaM-AI, PMA and ionomycin. As predicted, the IC_{50} of CsA, in the absence of ionomycin, decreases. In a typical experiment, the IC_{50} is reduced from 5 ng ml^{-1} to 3 ng ml^{-1} (Fig. 3*e*).

Rapamycin antagonizes the immunosuppressive effects of FK-506[9], does not inhibit calcineurin *in vitro*[3], and has no effect on ΔCaM-AI-dependent stimulation of the IL-2 promoter (Fig. 2*a*). To relate the FK-506 inhibition described above to known pharmacological characteristics of FK-506, we examined the ability of rapamycin to antagonize FK-506 in the presence of ΔCaM-AI. Typically, the 50% effective dose (ED_{50}) of rapamycin decreased three-fold in the presence of ΔCaM-AI (Fig. 3*f*), consistent with the hypothesis that the change in IC_{50} for each immunosuppressant results from increasing the concentration of calcineurin.

Several genes encoding catalytic subunits of calcineurin have been identified[10]. This study indicates that a truncated form of the type 1 (or α) gene acts in synergy with PMA to activate the IL-2 promoter. Our results provide the first biological evidence that calcineurin is an effector of Ca^{2+}-dependent transcriptional activation and is the FK-506-sensitive component of the TCR signal transduction pathway. In addition to IL-2 promoter activation in T cells, calcineurin may play a role in other Ca^{2+}-dependent, FK-506-sensitive events such as neutrophil degranulation[11], inhibition of Ly-6E gene transcription[12] and various CsA/FK-506 toxicities in other organs. □

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