at the same level as CS-transfected L cells and more efficiently than L cells incubated with peptide 370–390 (Fig. 4), indicating that the full T cell epitope was contained within residues 368–390. The observation that CTL recognized peptide 368–390 to the same degree as the transfectant gives a further indication that B10.BR CS-specific-CTL recognize only this single epitope on the CS protein. Peptide 368–390, however, represents a polymorphic region of the molecule[5,14–16]. This peptide epitope was also recognized by CTL generated from sporozoite-immunized mice but not by spleen cells from non-immunized mice stimulated by the transfectant in either the presence or absence of interleukin-2 (data not shown).

Our data demonstrate that malaria sporozoites can induce CS-protein-specific CTL, and that in B10.BR mice the CTL response is restricted to a single peptide epitope from a variant region of the protein. The implications of these results for the development of a malaria vaccine for use in humans are not encouraging. Although T cell responsiveness shows some variation, both with species and MHC haplotype, and human CTL epitopes on the CS protein have not yet been identified, studies of $CD4^+$ T cell epitopes on the CS protein suggest that those of mouse and human closely overlap and that they are mostly found in variant regions of the molecule (refs 7, 12 and M.F. Good *et al.*, unpublished observations). It seems likely that selective pressure for evasion of T cell recognition has led to the restricted number and polymorphic nature of T cell recognition sites on the CS protein. If human CTL recognition of the CS protein does prove to be restricted to variant epitopes, it may be critical for vaccine development to locate other sporozoite or hepatic stage proteins which contain non-polymorphic $CD8^+$ T cell recognition sites.

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Deactivation of macrophages by transforming growth factor-β

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Macrophage activation—enhanced capacity to kill, in a cell that otherwise mostly scavenges—is essential for host survival from infection and contributes to containment of tumours. Both microbes and tumour cells, therefore, may be under pressure to inhibit or reverse the activation of macrophages. This reasoning led to the demonstration of macrophage deactivating factors from both microbes[1,2] and tumour cells[3–5]. In some circumstances the host itself probably requires the ability to deactivate macrophages. Macrophages are essential to the healing of wounds and repair of tissues damaged by inflammation. Yet the cytotoxic products of the activated macrophages can damage endothelium, fibroblasts, smooth muscle and parenchymal cells (reviewed in ref. 6). Thus, after an inflammatory site has been sterilized, the impact of macrophage activation on the host might shift from benefit to detriment. These concepts led us to search for macrophage deactivating effects among polypeptide growth factors that regulate angiogenesis, fibrogenesis and other aspects of tissue repair. Among 11 such factors, two proteins that are 71% similar proved to be potent macrophage deactivators: these are transforming growth factor-β1 (TGF-β1) and TGF-β2.

Growth factors were tested over a broad concentration range for their ability to suppress the capacity of activated mouse peritoneal macrophages to release H_2O_2. The capacity to release H_2O_2 is a close biochemical correlate of macrophage activation, due to the prominent involvement of reactive oxygen intermediates in their antimicrobial function[7]. Activated macrophages were collected four days after intraperitoneal injection of sodium caseinate and plated at $1.2–1.3 \times 10^5$ per well in 96-well trays in Eagle's minimum essential medium (α-variant) with 10% horse serum (complete medium). Nonadherent cells were washed off at 2 hours and test media added to triplicate wells for the times indicated before the media were flicked out and the plates washed in saline. H_2O_2 release was then measured in the absence of cytokines over 90 min in response to 167-nM phorbol myristate acetate (PMA) by the horseradish peroxidase-catalysed oxidation of fluorescent scopoletin, and related to the protein content in the same walls, as described[8].

Incubation of macrophages for two days in 0.04–100 ng ml^{-1} of TGF-β1 suppressed H_2O_2 release by $86 \pm 11\%$ (mean ± s.e.m., $n = 83$ in 25 experiments). Macrophages incubated in medium alone released 243 ± 65 nmol H_2O_2 per mg cell protein per 90 min ($n = 33$ in 25 experiments). Similar results were obtained with TGF-β2, although ~8-fold higher concentrations were required. The concentrations causing 50% inhibition of H_2O_2 releasing capacity (EC_{50}) were 0.6 pM for TGF-β1 and 4.8 pM for TGF-β2 (Fig. 1), suggesting a physiological role for this action of the cytokines. In contrast, the following growth factors suppressed macrophage H_2O_2 releasing capacity by −21% to 30%, when tested over a ≥10,000-fold dose range up to 100 ng ml^{-1} (Fig. 1): natural mouse nerve growth factor, natural murine interleukin (IL)-3, recombinant human IL-1β, natural bovine fibroblast growth factor a and b, natural porcine platelet-derived growth factor, recombinant mouse colony stimulating factor (CSF) for granulocytes and macrophages, recombinant human CSF for granulocytes, and natural mouse epidermal growth factor (which shares receptors with TGF-α).

Deactivation induced by TGF-β1 or TGF-β2 was not evident over the first nine hours of incubation, but became half-maximal by 21-23 h at concentrations of 1–10 ng ml^{-1}, and by ~48 h for 0.1 ng ml^{-1} (Fig. 2). This suggests that TGF-β did not deactivate macrophages by triggering their respiratory burst, because exhaustion of respiratory burst capacity by triggering agents is evident immediately after the burst ceases (~1.5–3.5 h). Moreover, TGF-β1 at 1–100 ng ml^{-1} did not elicit H_2O_2 release

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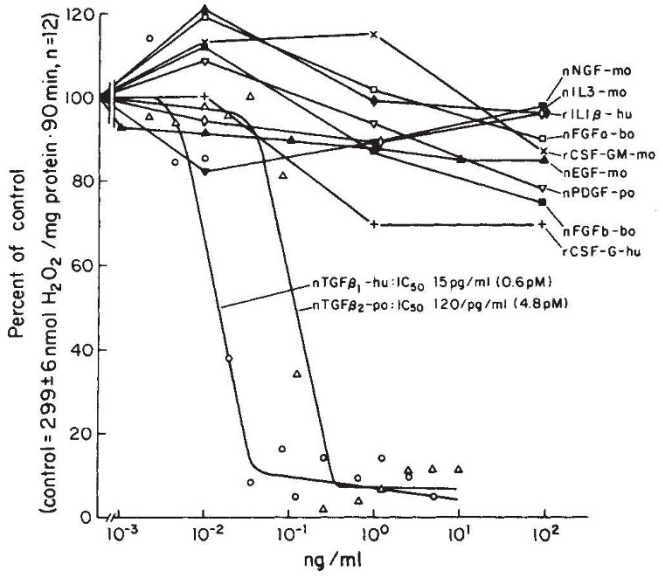

Fig. 1 Suppression of the H_2O_2 releasing capacity of macrophages following incubation for two days in TGF-β1 and TGF-β2, but not any of nine other polypeptide growth factors. Caseinate-elicited mouse peritoneal macrophages were incubated in the indicated concentrations of cytokines before being washed and challenged with 167-nM PMA to trigger secretion of H_2O_2. One of three similar experiments is shown (two for EGF). For clarity, only means are presented; s.e.m.s for the triplicates averaged 7.5% of the means; n = natural; r = recombinant; suffixes designate species of origin. All reagents were pure, or in the case of IL-3, partially purified. TGF-β1 was prepared from human platelets as described in ref. 24, except that the urea was removed by desalting on C18 HPLC (high pressure liquid chromatography). TGF-β2 (ref. 25) was purchased from R&D Systems, Minneapolis. Nerve growth factor (NGF) from mouse submaxillary gland was isolated by Dr Joshua Adler, and provided by Dr Moses Chao, both of Cornell University Medical College. Interleukin-3 (IL-3) (Genzyme, Boston, Massachusetts) was from the conditioned medium of LBRM-33-5A4 T lymphoma cells. Fibroblast growth factors (FGF)-acidic (a) and basic (b) from bovine brain and platelet derived growth factor (PDGF) were from Peninsula Laboratories, Belmont, California. Biologically active epidermal growth factor (EGF) from mouse salivary gland was purified in the laboratory of one of us (M.S.). Colony-stimulating factor-granulocytes/macrophages (CSF-GM) was from Immunex, Seattle, Washington. CSF-granulocytes (CSF-G) was from Amgen, Thousand Oaks, California.

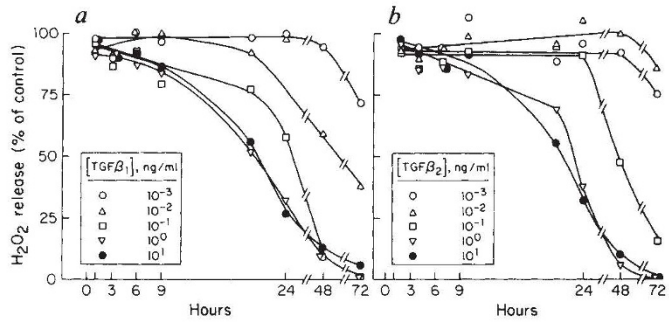

Fig. 2 Time course for suppression of H_2O_2 releasing capacity by (*a*) TGF-β1 or (*b*) TGF-β2. After incubation for the periods indicated in 0.001–10 ng ml^{-1} TGF-β, the macrophages were washed and challenged with 167-nM PMA to measure H_2O_2 release (nmol per mg protein per 90 min). Values are expressed as a per cent of the results for controls incubated for the same time periods without TGF-β. The control values were nearly constant over three days. The mean ±s.e.m. for the means of eight control triplicates was 372 ± 13 in *a* and 362 ± 19 in *b*. One of two similar experiments is shown.

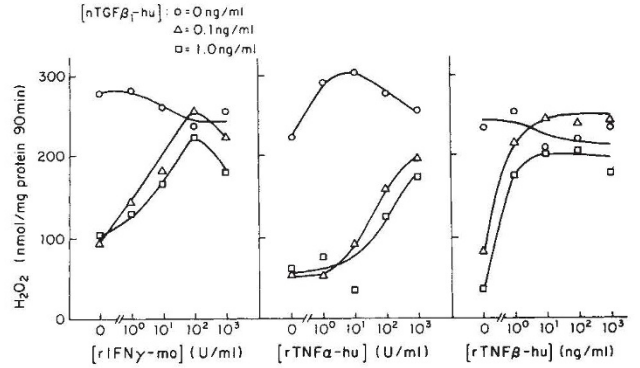

Fig. 3 Prevention of TGF-β1-induced deactivation by coincubation in macrophage activating factors. Activated macrophages were exposed to the following cytokines alone or in the indicated combinations: TGF-β1, and one of three pure recombinant proteins (all expressed in *Escherichia coli* and provided by Genentech)—*a*, murine interferon-γ (rIFNγ-mo, specific activity 5×10^7 U mg^{-1}), *b*, tumour necrosis factor-α-hu (TNFα, specific activity 3.6×10^7 U mg^{-1}) and (*c*) TNF-β-hu (lymphotoxin, specific activity 1.2×10^8 U mg^{-1}). After two days, the cells were washed and challenged with PMA. TGF-β1 concentrations were 0 (○), 0.1 (△), or 1 (□) ng ml^{-1}. Concentrations of the other cytokines are indicated on the abscissa. One of four similar experiments is shown.

from macrophages when added directly to the scopoletin assay.

To determine whether the state of macrophage activation reflects the balance between activating and deactivating factors, macrophages were incubated in TGF-β1 alone, macrophage activating factors alone, or combinations of these cytokines (Fig. 3). Suppression of H_2O_2 releasing capacity caused by TGF-β1 could be overcome by interferon-γ, tumour necrosis factor (TNF)-α, or TNF-β, at concentrations similar to those required to activate resident peritoneal macrophages[2,9,10].

Suppression of H_2O_2 releasing capacity by TGF-β did not reflect toxicity to the macrophages. Thus, ≥95% of macrophages excluded trypan blue after incubation for two days in ≥1-ng ml^{-1} TGF-β. Macrophages incubated for two days in 1- or 10-ng ml^{-1} TGF-β retained a capacity for extensive phagocytosis: treated macrophages took up ~20–25 starch granules per cell (each granule 2.6 μm in diameter), marginally less than control cells, as assessed by a method optimized to detect differences in maximal phagocytic capacity[11] (Fig. 4). But TGF-β did cause macrophages to become less tightly adherent to the plastic culture surface. This was reflected by a dose-dependent decrease in the amount of cell protein remaining after vigorous washing (IC_{50} ~ 0.8 pM for TGF-β1, ~5.2 pM for TGF-β2). Overall, after treatment for two days with 0.04–100 ng ml^{-1} TGF-β1, the adherent cell protein in vigorously washed monolayers averaged 59 ± 20% of control ($n = 83$ in 25 experiments).

Prominent sources of TGF-β include degranulating platelets, endothelial cells, fibroblasts, keratinocytes, tumour cells and T cells responding to antigen[12–16]. Thus, wounds, tumours[17] and T cells may all be able to restrain or reverse macrophage activation through the action of TGF-β. Eukaryotic cells can deactivate macrophages by other routes as well[3–5]. But the previously described macrophage deactivating factor from tumours[3–5] is immunochemically and functionally distinct from TGF-β (manuscript in preparation). Macrophages themselves can secrete TGF-β upon exposure to bacterial lipopolysaccharide[18]. This suggests the possibility of a negative feedback that may counter the potential for positive feedback implicit in the ability of the macrophage to produce some of its own activating factors, such as TNF-α[9,10] and CSF-GM[19].

Other reported actions of TGF-β1 on mononuclear phagocytes are both stimulatory and suppressive. TGF-β1

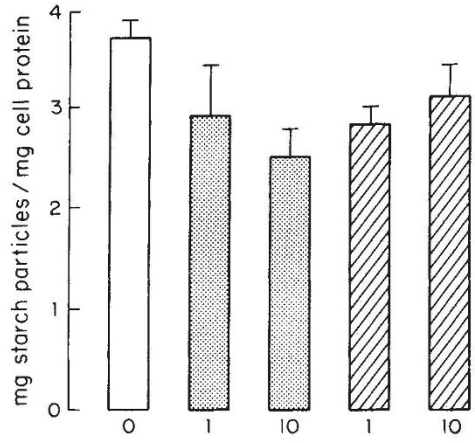

Fig. 4 Preservation of phagocytic function after deactivation of macrophages by TGF-β1 or TGF-β2. Macrophages (8.5×10^5) were plated on 13-mm glass coverslips and nonadherent cells removed after 2 h. Test media were added for 2 days as indicated: complete medium alone (open bar), or complete medium containing TGF-β1 (stippled bars) or TGF-β2 (hatched bars) at the concentrations indicated in ng ml^{-1}. After 2 days, macrophages were washed and incubated in complete medium for 2 h with 2 mg per coverslip of ^{14}C-acetylated starch granules isolated from seeds of *Amaranthus caudatus*. These conditions optimize the quantification of maximal phagocytic capacity by macrophages[11]. The coverslips were washed and the monolayers solubilized to determine the mg particles phagocytized per mg cell protein. Means ±s.e.m. of triplicates are shown.

induces chemotaxis[20,21] (EO_{50}, 0.004 pM)[20], release of fibroblast growth factors and accumulation of IL-1 mRNA (EC_{50}, ~40 pM)[20], and release of angiogenic factors[21]. On the other hand, exposure of macrophages to TGF-β1 is associated with ~50% suppression of TNFα release and ~35% suppression of Ia antigen expression (EC_{50}, 40–400 pM) (ref. 21 and personal communication, C. Czarniecki). Above, we have described virtually complete suppression of macrophage respiratory burst capacity by TGF-β1 (EC_{50}, 0.6 pM) and TGF-β2 (EC_{50}, 4.8 pM). These effects may reflect a coordinated response in wound healing[23], in which macrophages are recruited to scavenge debris and foster the growth of fibroblasts and endothelial cells, while being suppressed in their capacity for a respiratory burst that could be inimical to these cells.

The ability of TGF-β1 and TGF-β2 to ablate the respiratory burst of macrophages raises the possibility that these agents might have a role in the treatment of inflammatory disorders involving excessive macrophage activation.

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A gene induced by the plant hormone abscisic acid in response to water stress encodes a glycine-rich protein

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Plant hormones such as abscisic acid (ABA) appear to modulate the responses of plants under adverse conditions[1,2] ABA has a poorly-understood role in embryogenesis, accumulating in the stages before dessication[3,4], and altering the rate of transcription of a specific set of genes[5,6]. The functions of the proteins encoded by these genes, however, are unknown, and their messenger RNAs decrease again during early germination[7–9]. No correlation has been established between ABA levels and the induction of particular genes in non-embryonic organs. The level of ABA increases substantially in leaf tissues subjected to water stress[10] and thus it has been proposed that ABA mediates plant–water relations[1,10]. Here we describe the isolation of complementary DNA and genomic clones of a gene that is ABA-inducible in the maize embryo, and whose messenger RNA accumulates in epidermial cells, which is also induced by water stress and wounding in leaves. The deduced protein is rich in glycine. Identification of this gene will contribute to our understanding of the role of ABA.

After a series of differential screenings of a cDNA library constructed from maize dry embryo using cDNA synthesized from immature embryo poly(A)$^+$ RNA, with or without ABA treatment, six non cross-hybridizing clones were selected. These were shown to correspond to mRNAs present in dry embryos by Northern analysis, and their level increased precociously after ABA treatment of immature maize embryos. One of them (clone pMAH9) had an insert of 732 base pairs (bp) and hybridized to an RNA band of approximately the same size (Fig. 3). This clone was chosen for further study, and was used to screen a genomic library. The identity of a hybridizing genomic clone was confirmed by restriction mapping.

The sequences of the cDNA and genomic clones are shown in Fig. 1. They show perfect identity except for an insertion of 146 bp in the genomic sequence with the sequence features of an intron. There is a TATA box at the 5′-end. Only one plausible open reading frame was identified in the cDNA, and the sequence of the putative protein is shown in Fig. 1. The encoded protein is 157 amino acids long with a predicted relative molecular mass of 15,427 and an isoelectric point of 5.7. These values correspond closely with polypeptides detected in hybrid-released experiments using the pMAH9 plasmid (results not shown).

The protein sequence has a well-defined domain structure, clearly seen in hydrophilicity plots (Fig. 2). The first half of the sequence (residues 1–88) is composed of alternating α and short

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