CD3-coated tissue culture dishes. After 16 h, cells were gently detached, rested for 3–5 h on normal tissue-culture dishes, and then activated for various durations on 96-well plates containing co-immobilized anti-CD3 and anti-CD28 (5 μg ml^{-1} each). Proteins were extracted by the addition of an equal volume of 2× reducing sample buffer and, after being boiled, (0.5–1.0) × 10^6 cell equivalents were added to the lanes of 10% polyacrylamide gels. Proteins were transferred to Immobilon-P (Millipore) and probed with phospho-specific MAP kinase antibodies for detection by chemiluminescence (Pierce).

Nuclear extract preparation and electrophoretic mobility-shift analysis

Purified T cells (10^6) were washed in PBS and resuspended in 1 ml of 10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA and Complete protease inhibitors (Roche). The cells were incubated on ice for 15 min. Nonidet P40 was added to a final concentration of 0.5%, the cells were vortex-mixed vigorously, and the mixture was centrifuged for 30 s. The nuclear pellets were resuspended in 40 μl of 20 mM HEPES pH 7.9, 0.4 M NaCl, 1 mM EDTA and protease inhibitors, and the tube was rocked for 15 min at 4 °C. After centrifugation for 5 min, the supernatant was collected and glycerol was added to a final concentration of 50%; 2 μl of each extract was subjected to bandshift analysis. Electrophoretic mobility-shift analyses (EMSAs) were performed with the following oligonucleotides and binding buffers: NFAT oligonucleotide: 5′-GCCCAAAGAGGAAAA TTTGTTTCATACAG-3′; NFAT 5× binding buffer: 50 mM Tris-HCl pH 7.5, 500 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM EDTA, 50% glycerol, 250 μg ml^{-1} poly (dI:dC), 5 mM dithiothreitol and 1 mg ml^{-1} BSA; NF-κB oligonucleotide: 5′-ACCAAGAGGGATTTCACCT AAATC-3′; NF-κB 5× binding buffer: 25 mM Tris-HCl pH 7.5, 5 mM EDTA, 250 μg ml^{-1} poly (dI:dC), 5 mM dithiothreitol and 1 mg ml^{-1} BSA; AP-1 oligonucleotide: 5′-CGCTT GATGACTCAGCCGGAA-3′; AP-1 5× binding buffer: same as that for NFAT. 5 × 10^5 c.p.m. of labelled probe was used in each reaction, and bandshifts were resolved on 5% polyacrylamide gels in 0.5 × TBE running buffer.

Immunizations

KLH (20 μg ml^{-1}) was allowed to adsorb on 1 mg of aluminium hydroxide for 1 h at room temperature. After washing unadsorbed KLH with PBS, the KLH–aluminium hydroxide mixture was resuspended in 200 μl of PBS and injected intraperitoneally. Splenocytes recovered 2 weeks later were stimulated at 2 × 10^5 cells per well (in triplicate) with 0, 0.1, 1 or 10 μg ml^{-1} KLH for 3 d. Cells were then pulsed with 1 μCi of [^{3}H]thymidine for 1 d.

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Control of T_H2 polarization by the chemokine monocyte chemoattractant protein-1

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Activated T lymphocytes differentiate into effector cells tailored to meet disparate challenges to host integrity[1]. For example, type 1 and type 2 helper (T_H1 and T_H2) cells secrete cytokines that enhance cell-mediated and humoral immunity, respectively. The chemokine monocyte chemoattractant protein-1 (MCP-1) can stimulate interleukin-4 production[2] and its overexpression is associated with defects in cell-mediated immunity[3], indicating that it might be involved in T_H2 polarization. Here we show that MCP-1-deficient mice are unable to mount T_H2 responses. Lymph node cells from immunized *MCP-1*$^{-/-}$ mice synthesize extremely low levels of interleukin-4, interleukin-5 and interleukin-10, but normal amounts of interferon-γ and interleukin-2. Consequently, these mice do not accomplish the immunoglobulin subclass switch that is characteristic of T_H2 responses and are resistant to *Leishmania major*. These effects are direct rather than due to abnormal cell migration, because the trafficking of naive T cells is undisturbed in *MCP-1*$^{-/-}$ mice despite the presence of MCP-1-expressing cells in secondary lymphoid organs of wild-type mice. Thus, MCP-1 influences both innate immunity, through effects on monocytes, and adaptive immunity, through control of T helper cell polarization.

MCP-1 is a CC chemokine that attracts monocytes, memory T lymphocytes and natural killer cells *in vitro*[4–8]. Several models of transgenic expression in the mouse have validated it as a predominantly monocytic chemoattractant *in vivo*[3,9–11]. Despite the existence of other CC chemokines that attract monocytes *in vitro* with

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the same potency, MCP-1-deficient mice demonstrate that it is solely responsible for monocyte recruitment in several inflammatory settings[12]. There is similar evidence for an essential pathogenetic role of MCP-1 in disease models such as atherosclerosis[13,14] and asthma[15], in which MCP-1 is required for the accumulation of macrophages in diseased tissue. Because of these findings and the observation that inflammatory stimuli induce MCP-1 expression, MCP-1 has been considered to be an inflammatory chemokine that has little effect on adaptive immunity or immune-cell trafficking.

However, several lines of evidence indicate that MCP-1 might influence T-cell immunity. First, T lymphocytes express the MCP-1 receptor CCR2 (ref. 16), and MCP-1 is a potent chemoattractant for memory T cells *in vitro*[6,16] (although T cells are nearly absent from transgenic MCP-1-induced infiltrates *in vivo*). Second, MCP-1 expression is associated with the development of polarized T_H2 responses[12,17], and MCP-1 enhances the secretion of interleukin-4 (IL-4) by T cells[2,18]. Third, in immune-mediated diseases with a T_H2 character, such as asthma, MCP-1 is expressed at high levels and its neutralization in animal models ameliorates disease[15]. Last, other chemokines and their receptors are linked to specific responses of T-helper cells[19,20]. These observations prompted our analysis of MCP-1's influence on the development of polarized immune responses *in vivo*.

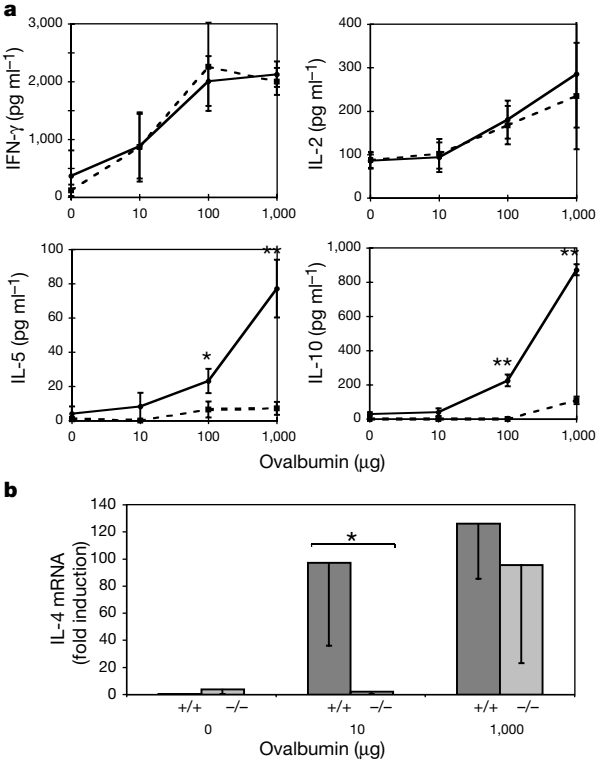

Figure 1 Effect of MCP-1 on cytokine synthesis. **a**, Seven days after immunization with TNP-Ova, single-cell suspensions were prepared from draining lymph nodes of *MCP-1*$^{+/+}$ and *MCP-1*$^{-/-}$ mice. Cells were challenged *in vitro* with increasing concentrations of ovalbumin, and concentrations of the indicated cytokines were determined by ELISA every 24 h for 3 d. Dose responses are shown for the day on which the highest cytokine levels were observed (day 2 for interferon-γ and IL-2, day 3 for IL-5 and IL-10). Values are means for 6 mice per group and are representative of two independently performed experiments. Solid line, *MCP-1*$^{+/+}$; dotted line, *MCP-1*$^{-/-}$. Asterisk, $P < 0.05$ compared with *MCP-1*$^{-/-}$ mice; two asterisks, $P < 0.005$ compared with *MCP-1*$^{-/-}$ mice. **b**, Cells from *MCP-1*$^{+/+}$ (+/+) or *MCP-1*$^{-/-}$ (–/–) mice were challenged *in vitro* with the indicated concentrations of ovalbumin for 3 d, and IL-4 mRNA content was determined by quantitative PCR and normalized to GAPDH mRNA levels. Induction is normalized to untreated *MCP-1*$^{+/+}$ cells, and values represent the means of 3 mice per group. Asterisk, $P = 0.05$ for the difference between *MCP-1*$^{+/+}$ and *MCP-1*$^{-/-}$ mice.

To examine the potential for *MCP-1*$^{-/-}$ mice to develop T_H2 responses, we immunized mice with trinitrophenol-derivatized ovalbumin (TNP-Ova) in incomplete Freund's adjuvant. One week later, draining lymph nodes were isolated and single-cell suspensions were challenged with increasing concentrations of ovalbumin. Figure 1a shows that cells from *MCP-1*$^{+/+}$ and *MCP-1*$^{-/-}$ mice secreted the same amounts of interferon-γ and IL-2, suggesting that the T_H1 component of this response was intact. Consistent with that observation was the finding that IL-12 levels were the same in supernatants from both cultures (~30 pg ml^{-1}). In contrast, cells from *MCP-1*$^{-/-}$ mice secreted extremely low levels of IL-5 and IL-10, whereas cells from wild-type mice secreted substantial amounts.

Because lymph node cells from immunized wild-type C57Bl/6 mice secreted undetectable amounts of IL-4, we measured the expression of IL-4 messenger RNA by polymerase chain reaction with reverse transcription (RT–PCR) (Fig. 1b). Quantitative analysis showed that *MCP-1*$^{-/-}$ mice expressed 50-fold less IL-4 messenger RNA than did wild-type mice in response to intermediate doses of ovalbumin (10 μg ml^{-1}). At higher ovalbumin challenge doses (1000 μg ml^{-1}), however, this deficiency could be overcome, which was not true of IL-5 and IL-10 (Fig. 1a), suggesting the possibility that MCP-1's effects on T_H2 polarization may be partially independent of IL-4.

We examined the physiological consequences of this deficiency in cytokine secretion by testing whether or not *MCP-1*$^{-/-}$ mice could accomplish immunoglobulin subclass switching characteristic of T_H2 responses. First, however, Fig. 2a shows that the ratio of total serum IgG_1 to IgG_{2a} in naive *MCP-1*$^{-/-}$ mice was already decreased 10-fold as compared with wild-type mice. The ratio of total IgG_1 to IgG_{2b} was decreased twofold, which, although a less substantial difference, was still highly significant. These altered ratios were due both to decreases in IgG_1 and to increases in IgG_{2a} and IgG_{2b} in *MCP-1*$^{-/-}$ mice as compared with wild-type mice. Thus, in the

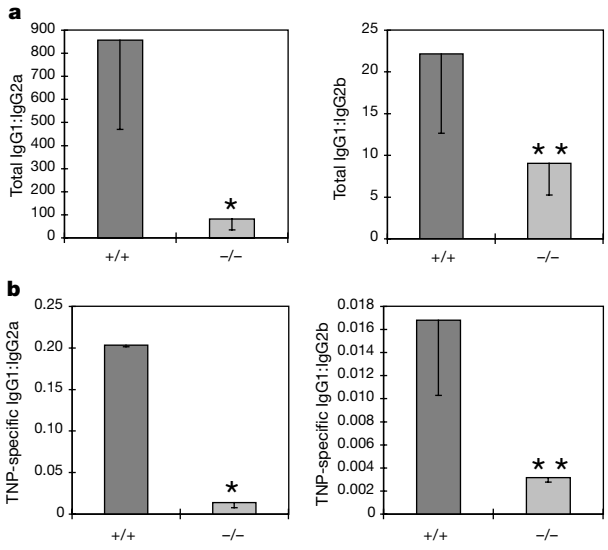

Figure 2 Effect of MCP-1 on IgG subclass concentrations. **a**, Serum was collected from *MCP-1*$^{+/+}$ (+/+) and *MCP-1*$^{-/-}$ (–/–) mice, and total IgG subclass concentrations were determined by ELISA. To normalize for variation between animals, the data are shown as ratios of IgG_1 to IgG_{2a} and IgG_1 to IgG_{2b}. Means were derived from three or four animals in each group; these data are typical of two independently performed experiments. Asterisk, $P < 0.008$ compared with *MCP-1*$^{+/+}$ mice; two asterisks, $P < 0.05$ compared with *MCP-1*$^{+/+}$ mice. **b**, TNP-specific IgG subclass ratios were determined in mice 7 d after immunization with TNP-Ova. Means were derived from three or four animals in each group; these data are typical of two independently performed experiments. Asterisk, $P < 2 \times 10^{-7}$ compared with *MCP-1*$^{+/+}$ mice; two asterisks, $P < 0.008$ compared with *MCP-1*$^{+/+}$ mice.

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absence of MCP-1, mice have total immunoglobulin subclass concentrations indicative of a baseline T_H1 bias. After immunization, the T_H1-skewed subclass ratios observed in naive mice persisted in TNP-specific immunoglobulin (Fig. 2b). The ratio of TNP-specific IgG_1 to IgG_{2a} was decreased 10-fold and the ratio of TNP-specific IgG_1 to IgG_{2b} was decreased 4-fold in *MCP-1*$^{-/-}$ mice in comparison with wild-type mice. Because IL-4 stimulates the IgG_1 switch and inhibits the IgG_{2a} and IgG_{2b} switch, the fact that high concentrations of antigen could overcome the IL-4 mRNA blockade *in vitro* (Fig. 2b) seems to be physiologically irrelevant in this setting.

To support further the notion that *MCP-1*$^{-/-}$ mice are T_H2 deficient, we tested their susceptibility to infection by *Leishmania major*. Because Balb/c mice mount a T_H2 response to this parasite, they succumb to infection. By 7 weeks after the injection of 2×10^6 promastigotes in the hind footpad, *MCP-1*$^{-/-}$ Balb/c mice had significantly less footpad swelling than wild-type Balb/c mice (Fig. 3) and their lesions were stabilizing, whereas those in wild-type mice continued to progress to the point that it was necessary to kill many of the wild-type controls by 9–10 weeks. However, lesions in *MCP-1*$^{-/-}$ mice had not yet healed by this time, suggesting that their phenotype was intermediate between completely susceptible and completely resistant strains.

Although the development of a T_H2 response could be due to a direct effect of MCP-1 on T cells, it is also possible that MCP-1 attracts precursor cells to a microenvironment containing a mixture of cells and cytokines that are actually responsible for T_H2 polarization. In that case, naive precursor cells might require MCP-1 if they are to travel to T-cell zones in secondary lymphoid organs where they encounter antigen. To test this idea, we isolated naive T lymphocytes from a congenic mouse strain carrying the Ly-5.1 (CD45.1) allele and injected them intravenously into wild-type C57Bl/6 or *MCP-1*$^{-/-}$ mice in a C57Bl/6 background, which carry the Ly-5.2 (CD45.2) allele. Five hours later, exogenous T cells migrating to the T-cell zones of spleens and lymph nodes were identified by using anti-Ly-5.1 (Fig. 4a). Image analysis showed that the same numbers of exogenous T cells had accumulated in the T-cell zones of wild-type and *MCP-1*$^{-/-}$ mice (Fig. 4b), indicating the absence of a T-cell trafficking defect in *MCP-1*$^{-/-}$ mice.

Nevertheless, *in situ* hybridization revealed the presence of cells in secondary lymphoid organs that constitutively express MCP-1. Figure 5 shows that scattered cells in the splenic periarteriolar lymphatic sheaths (PALS) expressed MCP-1. In lymph nodes, a similarly scattered distribution of MCP-1-expressing cells was found in the medulla (Fig. 5f). Seven days after immunization, there was a general increase in low-level MCP-1 expression throughout the PALS, and cells expressing higher levels of MCP-1 extended farther from the arteriole (Fig. 5d). Although we have not yet identified the cell types responsible for this expression, they are clearly localized to the T-cell zone and do not have the morphology of macrophages or dendritic cells. There are therefore MCP-1-expressing cells within secondary lymphoid organs that are in locations appropriate for taking a role in the direct stimulation of polarized T-cell responses.

Despite MCP-1's effects on monocytes and macrophages, which might have implied a role in T_H1-polarized responses, our data indicate instead that it is required for the development of T_H2 responses. The resistance of *MCP-1*$^{-/-}$ mice to *Mycobacterium tuberculosis*[12] is consistent with their fully functional T_H1 responses. The severe T_H2 defect in *MCP-1*$^{-/-}$ mice is unlikely to be due to adventitious compensatory changes in response to *MCP-1* disruption because animals made acutely MCP-1-deficient by using neutralizing antibodies also had difficulty in mounting full T_H2 responses[17]. Nevertheless, this deficiency contrasts with a reported T_H1 defect in *CCR2*$^{-/-}$ mice, which lack the only receptor for MCP-1 that has been cloned so far[21,22]. This discrepancy might not be surprising given the fact that CCR2 has at least two additional high-affinity ligands in the mouse (MCP-3 and MCP-5), which, in an appropriate context, might stimulate T_H1 polarization. Furthermore, there is biological evidence for another MCP-1 receptor[23], and the receptor known as D6 binds MCP-1, although it does not signal[24]. MCP-1 might exert its effects on T_H2 polarization through these receptors rather than CCR2. Lastly, the chemokine receptor CCR4 has been reported to bind MCP-1 with high affinity[25].

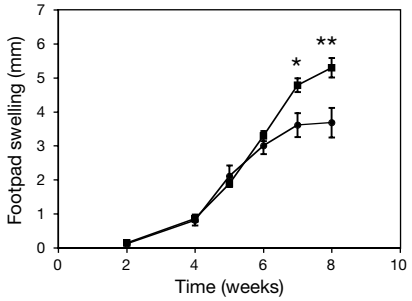

Figure 3 Resistance of *MCP-1*$^{-/-}$ mice to *Leishmania major*. Promastigotes (2×10^6) were injected into the right hind footpads of six wild-type Balb/c mice and eight *MCP-1*$^{-/-}$ mice in a Balb/c background. Right and left footpad thicknesses were measured weekly and footpad swelling was determined as the difference between right and left footpad thicknesses. Circles, wild-type; squares, *MCP-1*$^{-/-}$; bars indicate s.e.m. Asterisk, $P = 0.05$ compared with wild-type mice; two asterisks, $P = 0.007$ compared with wild-type mice.

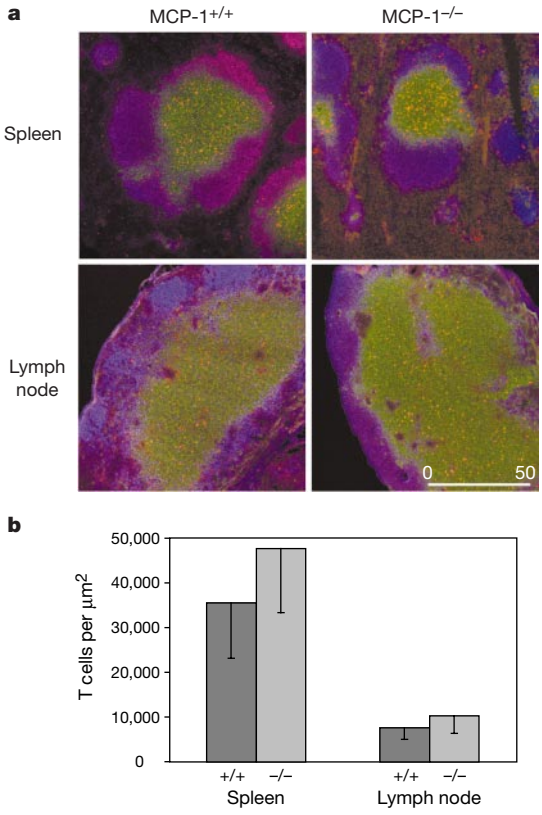

Figure 4 Effect of MCP-1 on T-lymphocyte trafficking. **a**, Purified T lymphocytes from wild-type CD45.1$^+$ (Ly-5.1$^+$) mice were injected intravenously into CD45.2$^+$ (Ly-5.2$^+$) *MCP-1*$^{+/+}$ and *MCP-1*$^{-/-}$ mice. Five hours later, spleens and lymph nodes were isolated and processed for immunohistochemistry. B-cell areas are stained purple, T-cell areas are stained green, and injected T cells are stained red. Scale bar, 50 μm. **b**, The area of the T-cell zone and the number of injected T cells per μm^2 of T-cell zone were determined by image analysis of confocal microscopy images. Means and s.e.m. are shown for three wild-type and three *MCP-1*$^{-/-}$ mice.

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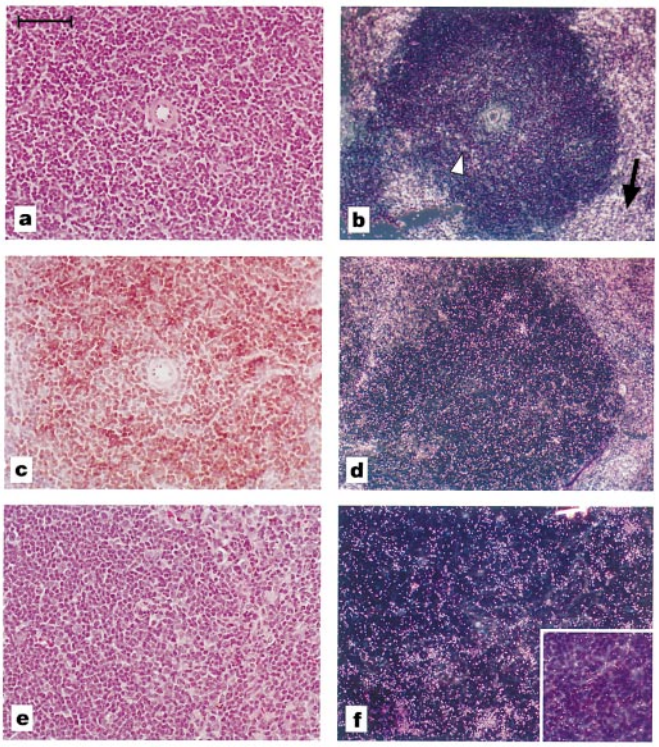

Figure 5 Expression of MCP-1 in spleen and lymph nodes. Spleens and lymph nodes from naive C57Bl/6 mice and C57Bl/6 mice 7 d after immunization were analysed for MCP-1 expression by *in situ* hybridization. **a**, Spleen from naive mouse stained with haematoxylin and eosin; **b**, spleen from naive mouse hybridized with antisense MCP-1 probe; **c**, spleen from naive mouse stained with anti-CD3; **d**, spleen from immunized mouse hybridized with antisense MCP-1 probe; **e**, lymph node from naive mouse stained with haematoxylin and eosin; **f**, lymph node from naive mouse hybridized with antisense MCP-1 probe (inset, serial section hybridized with sense MCP-1 probe). The white arrowhead in **b** indicates specific probe hybridization; the black arrow indicates areas of birefringence in the red pulp that did not show specific hybridization under bright-field examination. Panels **a**, **b**, **c**, **e** and **f** are serial sections. Hybridization of tissues from *MCP-1*$^{-/-}$ mice with the antisense probe showed no specific hybridization, indicating that the murine MCP-1 probe did not cross-hybridize with other chemokine mRNAs. Scale bar, 100 μm.

Although others have not reproduced this finding[26], CCR4 is preferentially expressed by T_H2 cells[19,20], and we cannot exclude the possibility that MCP-1 might produce some of its effects on T-cell polarization by using this receptor.

MCP-1's influence on memory/effector T-cell heterogeneity has important implications for pathogenesis. For example, MCP-1 is thought to have a pathobiological role in inflammatory disorders because of its ability to attract monocytes. However, neutralization of MCP-1 in rodent models of asthma, for example, accomplishes much more than simply preventing the appearance of macrophages in the air spaces[15]. It also reduces the total cellularity of the bronchoalveolar lavage fluid and lessens airway hyperreactivity. If MCP-1's sole activity were monocyte chemoattraction, its neutralization would be unlikely to have such wide-ranging effects. But, given its influence on T_H2 polarization, MCP-1 could affect both proximal (that is, immune T_H2-driven) and distal (that is, macrophage effector) steps in asthma pathogenesis. The same dual control of inflammatory pathology might occur in human asthma and in other diseases with high levels of MCP-1 expression, such as atherosclerosis or dementia associated with human immunodeficiency virus[13,27,28]. The present work shows that a single chemokine can affect two disparate and essential pathways in disease, making MCP-1 a very attractive target for anti-inflammatory drugs. □

Methods

Immunization

TNP-Ova was prepared by incubating a 5:1 mixture of 1% ovalbumin (Sigma) and 5% trinitrobenzenesulphonic acid (Sigma) at room temperature for 4 h, followed by extensive dialysis against PBS buffer. A total of 200 μg of TNP-Ova emulsified in incomplete Freund's adjuvant was injected subcutaneously in three to five sites on the back and in both hind footpads of wild-type C57Bl/6 mice or *MCP-1*$^{-/-}$ mice in a C57Bl/6 background.

Cytokine measurements

Single-cell suspensions were prepared from lymph nodes 7 d after immunization with TNP-Ova. Cells were added to multiwell tissue-culture plates at 2×10^6 cells ml^{-1} in RPMI 1640 supplemented with 10% fetal bovine serum. Ovalbumin was added to each well at the indicated concentration and supernatants were recovered every 24 h for 3 d. Cytokine concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (R&D Systems). For RT–PCR analysis, RNA was isolated by using RNeasy (Qiagen). Total RNA (2 μg) was reverse-transcribed and the cDNA quantified by real-time PCR with the TaqMan system (PE Corporation). Primer and probe sequences for IL-4 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are available on request. Values for IL-4 mRNA were normalized to the relative quantity of GAPDH mRNA in each sample.

IgG subclass measurements

To measure total IgG subclass concentrations, serum from naive or immunized mice was incubated in plates precoated with capturing antibodies specific for each subclass (PharMingen) for 2 h at room temperature. After five washes with PBS, 0.05% Tween 20, the appropriately matched biotinylated anti-subclass antibody was added to each well and incubated for 2 h at room temperature. After another five washes with PBS, 0.05% Tween 20, streptavidin-conjugated horseradish peroxidase was added, the plates were developed with 3-ethylbenzthiazoline-6-sulphonic acid and absorbance was read at 405 nm. Measurement of TNP-specific IgG subclass concentrations was performed in a similar manner except that the ELISA plates were precoated with TNP-BSA rather than capture antibodies.

Leishmania major infection

Promastigotes were provided by A. Satoskar and J. David (Harvard School of Public Health). *L. major* parasites (2×10^6) were injected into the hind footpads of 4–6-week-old male wild-type Balb/c mice or *MCP-1*$^{-/-}$ mice in a Balb/c background, and footpad thickness was measured weekly with a Peacock dial gauge.

T-cell trafficking.

T lymphocytes were isolated from lymph nodes and spleens of 8–12-week-old male C57Bl/6 mice congenic for CD45.1 (a gift from L. Clayton and E. Reinherz, Dana-Farber Cancer Institute) with a negative purification method (StemCell Technologies). Cell preparations (>95% T cells) were adjusted to 2×10^7 ml^{-1} and injected into the tail veins of

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8–12-week-old male wild-type or *MCP-1*$^{-/-}$ mice. Five hours after injection, spleens and lymph nodes were embedded in Tissue-Tek optimum cutting temperature compound (VWR) sectioned by cryostat and fixed in acetone. Sections were incubated for 1 h with Cychrome-conjugated rat anti-mouse CD45R/B220, fluorescein isothiocyanate-conjugated rat anti-mouse CD90.2 (Thy-1.2) and phycoerythrin-conjugated mouse anti-mouse CD45.1 (Ly-5.1), each diluted 1:100 in PBS (PharMingen). After three washes with PBS, 0.05% Tween 20, slides were mounted and examined by confocal microscopy. Image analysis was performed with Image Pro software (Media Cybernetics).

Immunohistochemistry and *in situ* hybridization

Spleens and lymph nodes were fixed in 4% paraformaldehyde in PBS and embedded in paraffin. Sections were processed for immunohistochemistry by using rat anti-human CD3, which crossreacts with murine CD3 (Harlan Bioproducts). A rat myeloma protein of identical isotype was used as a primary antibody control. Slides were incubated with biotinylated secondary antibodies and avidin–biotin-complexed horseradish peroxidase (Vector Laboratories), and developed with diaminobenzamidine. For *in situ* hybridization, probes were generated by *in vitro* transcription of pcJE-1 (ref. 29) in the presence of [^{35}S]UTP. Transcription, hybridization and development were performed as described[30].

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Betaglycan binds inhibin and can mediate functional antagonism of activin signalling

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Activins and inhibins[1], structurally related members of the TGF-β superfamily of growth and differentiation factors[2], are mutually antagonistic regulators of reproductive and other functions[1,3]. Activins bind specific type II receptor serine kinases (ActRII or IIB)[4–6] to promote the recruitment and phosphorylation of the type I receptor serine kinase, ALK4 (refs 7–9), which then regulates gene expression by activating Smad proteins[2]. Inhibins also bind type II activin receptors but do not recruit ALK4, providing a competitive model for the antagonism of activin by inhibin[9–11]. Inhibins fail to antagonize activin in some tissues and cells, however, suggesting that additional components are required for inhibin action[9,12,13]. Here we show that the type III TGF-β receptor, betaglycan[14,15], can function as an inhibin co-receptor with ActRII. Betaglycan binds inhibin with high affinity and enhances binding in cells co-expressing ActRII and betaglycan. Inhibin also forms crosslinked complexes with both recombinant and endogenously expressed betaglycan and ActRII. Finally, betaglycan confers inhibin sensitivity to cell lines that otherwise respond poorly to this hormone. The ability of betaglycan to facilitate inhibin antagonism of activin provides a variation on the emerging roles of proteoglycans as co-receptors modulating ligand–receptor sensitivity, selectivity and function[16–19].

Betaglycan binds TGF-β isoforms with high affinity and increases the functional interaction between TGF-β and its type II and type I signalling receptors[2,20]. While screening for potential inhibin-binding proteins, we detected inhibin binding in cells expressing the complementary DNA encoding betaglycan. Figure 1a shows that membranes from HEK 293 cells transfected with betaglycan exhibited specific, high-affinity inhibin binding (K_i = 0.6 (0.5–0.9) nM, whereas membranes from cells transfected with empty vector had undetectable specific inhibin binding. In competition binding assays, the inhibin-binding affinity was quite low in cells expressing ActRII alone K_i = 6.3 (2.9–13.4) nM, consistent with previous results[4], but increased approximately 30-fold in cells

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