Treatment of ongoing autoimmune encephalomyelitis with activated B-cell progenitors maturing into regulatory B cells

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The influence of signals perceived by immature B cells during their development in bone marrow on their subsequent functions as mature cells are poorly defined. Here, we show that bone marrow cells transiently stimulated in vivo or in vitro through the Toll-like receptor 9 generate proB cells (CpG-proBs) that interrupt experimental autoimmune encephalomyelitis (EAE) when transferred at the onset of clinical symptoms. Protection requires differentiation of CpG-proBs into mature B cells that home to reactive lymph nodes, where they trap T cells by releasing the CCR7 ligand, CCL19, and to inflamed central nervous system, where they locally limit immunopathogenesis through interleukin-10 production, thereby cooperatively inhibiting ongoing EAE. These data demonstrate that a transient inflammation at the environment, where proB cells develop, is sufficient to confer regulatory functions onto their mature B-cell progeny. In addition, these properties of CpG-proBs open interesting perspectives for cell therapy of autoimmune diseases.

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lymphocytes exert complex functions in autoimmune diseases. On the one hand they can promote these diseases, as shown by the beneficial effects of B-cell depletion therapies in rheumatoid arthritis or multiple sclerosis (MS)1,2. On the other hand, their negative regulatory functions can provide protection, as initially shown in models of ulcerative colitis3, experimental autoimmune encephalomyelitis (EAE)4 and collagen-induced arthritis5. More precisely, mice with an interleukin (IL)-10 deficiency restricted to B cells developed a severe chronic form of EAE, while those harbouring wild-type (WT) B cells rapidly recovered from disease6. The unique capacity of B cells to reduce the severity of autoimmune diseases through provision of IL-10 has kindled enormous interest in the identification of the responsible B-cell sub-populations, and the signals controlling their expression of suppressive functions. Several B-cell subsets can produce IL-10 on stimulation in vitro, and protect recipient mice from autoimmune disease on adoptive transfer. These include B10 cells with a CD5+ CD1dhigh phenotype, transitional type 2 (T2)-marginal zone precursors, and CD5+ B-1 cells (refs 7–9). Moreover, analysis of the cells producing IL-10 in a suppressive manner in vivo identified CD138high plasma cells residing either in spleen10 or LN11 as major IL-10 producers during EAE. In addition, IL-35 (ref. 10) and PD-L1 (ref. 12) were recently shown to mediate protection against EAE displayed by B regulatory cells.

Toll-like receptor (TLR) agonists are particularly important in this context because of their unique capacity to induce IL-10 expression in mature naive B cells, and the requirement for intrinsic TLR signalling in B cells for recovery from EAE13. Similarly, CD5+ CD1dhigh B cells depend on activation by TLR-4 or -9 agonists to produce IL-10 in vitro14. In addition to intrinsic TLR signalling, signals provided to B cells through the BCR or CD40 are also necessary to achieve a fully protective B-cell-mediated regulation15–19.

Human B cells can also produce IL-10, and evidence is accumulating that they can subsequently behave as negative regulators of immunity. Duddy et al.20 found that B cells from MS patients produce lower amounts of IL-10 on activation than their counterparts from healthy individuals, suggesting that MS is facilitated by a defect in this regulatory circuit. This defective IL-10 production was subsequently confirmed by Correale and Farez21 who observed that helminth infections could restore normal IL-10 production by B cells in this disease, which correlated with an improvement of the disease course compared with non-infected patients. These data suggest that promoting B-cell-mediated regulation might help to reduce MS progression. Of note, some MS treatments, such as interferon (IFN)-β22 or glatiramer acetate23,24 have been shown to enhance IL-10 secretion by B cells.

Although our knowledge of the peripheral B-cell subsets implicated in immune regulation, and the signals controlling their regulatory activity has greatly improved, we still have little data on whether B-cell development in bone marrow (BM) might influence the subsequent capacity of mature B cells to negatively regulate immunity in periphery. We recently gained some evidence of this by showing that proB cells emerging from BM cell cultures transiently stimulated with the TLR-9 agonist CpG prevented type 1 diabetes in NOD mice on adoptive transfer25. These cells, which we termed as CpG-proB cells, differentiated into various more mature B-cell subsets in recipient mice. We could demonstrate that they provided protection by reducing pathogenic IL-21 secretion by T cells, and by promoting apoptosis of T-effector cells25. However, it was difficult to further analyse how the administered CpG-proBs protected recipient mice from disease in this model due to the lack of mutant mouse strains on the NOD background. We therefore addressed the question, whether a similar subset emerged in the C57BL/6 mouse strain. To this end, we used the myelin oligodendrocyte glycoprotein (MOG)35–55-induced EAE model in which B-cell-mediated regulation has been extensively studied. So far described regulatory B-cell subsets only acted at the initiation phase of the disease, except PD-L1hi mature splenic B cells of which 1 million cells injected at day 7 after immunization provided protection against EAE12, albeit milder than when injected at day 0. However, we find that a single injection of only 60,000 CpG-proB cells at the onset of EAE clinical signs markedly reduces disease progression in recipient mice. In contrast, adoptive transfer of control non-stimulated proBs does not influence the disease course in recipient mice. This suppression requires the differentiation of the administered CpG-proB cells into mature B cells in the periphery and in the central nervous system (CNS). Collectively, these results shed light on the importance of the BCR-independent signals that B-cell progenitors receive in the BM environment to develop into mature B cells with regulatory rather than inflammatory functions. In addition, these properties of CpG-proBs open interesting perspectives for cell therapy of autoimmune diseases.

**Results**

ProB cells exposed to CpG protect against ongoing EAE. We previously found that on short-term incubation with CpG-1668 (CpG-B), BM cells from NOD mice gave rise to a population of pro-B cells that protected recipient mice from diabetes on adoptive transfer25. To assess whether a similar process took place in a strain of mice which are more amenable to genetic studies, we evaluated the effect of CpG-B on BM cells from C57BL/6J mice. This treatment led to emergence of cells expressing a c-kit+ Sca-1+ B220+ IgM– phenotype, and heterogeneous in terms of PDCA-1 expression (Fig. 1a and Supplementary Fig. 1). A similar BM population was observed in vivo in mice after ip injection of CpG-B, validating the use of in vitro cultures (Supplementary Fig. 2). The bright B220+ cells are gated out since they correspond to the more mature B cells contaminating the c-kit+ magnetically sorted cells. Moreover, since TLR-9 stimulation has been shown to promote deviation of hematopoiesis away from the B-cell lineage towards the PDCA-1+ plasmacytoid dendritic cell lineage26, B-cell precursors were further sorted by excluding the PDCA-1+ fraction (Fig. 1a). The resulting PDCA-1− population was closely related to the pro-B cell stage of differentiation, being CD19+ CD24hi IgM− CD11b− CD11c+, as well as expressing the IL-7Rz chain (CD127), CD43 and the transcription factor Pax5 (Fig. 1b and Supplementary Fig. 3a) characterizing B-cell lineage commitment. They all expressed CD1d, but were negative for CD5 (Fig. 1b). It is noteworthy that this effect was not restricted to TLR-9 agonists, because agonists of TLR-2, -4, -5, -6 and -7 induced development of a similar population, unlike agonists of TLR-1 and -3 (Fig. 1c). As expected, these cells did not appear in BM cell cultures from MyD88-deficient mice after incubation with CpG-B (Fig. 1c). Collectively, these data suggest that TLR agonists induce in vitro and in vivo the formation of a unique population of proB cells in BM from C57BL/6J mice, as previously found in NOD mice25.

We next examined whether these cells could protect recipient mice from EAE on adoptive transfer. Remarkably, a single injection of only 60,000 CpG-proBs (Fig. 1d) isolated either from in vitro BM cell culture activated with CpG (Fig. 1e, Table 1) or from BM of CpG-injected donors (Fig. 1f) to mice at the time of EAE onset (d12 after immunization) resulted in a marked attenuation of the disease course, relative to control mice that received only phosphate-buffered saline (PBS). Conversely, neither control pro-B cells isolated using their typical markers
Figure 1 | Phenotypic analysis of CpG-induced c-kit<sup>+</sup> Sca-1<sup>+</sup> B220<sup>+</sup> PDCA-1<sup>−</sup> IgM<sup>−</sup> BM cells and assessment of disease protection against ongoing EAE. (a) BM cells incubated for 18 h with CpG-B (1 μg ml<sup>−1</sup>) were magnetically selected for c-kit<sup>+</sup> cells, further labelled for Sca-1, B220, PDCA-1 and IgM and electronically sorted into small-size (FSC<sub>low</sub>SSC<sub>low</sub>) c-kit<sup>+</sup> Sca-1<sup>−</sup> B220<sup>+</sup> PDCA-1<sup>−</sup> IgM<sup>−</sup> cells. (b) Flow cytometry analysis of indicated B-cell markers expression by CpG-proB cells after cell-sorting as in a. (c) Cells were stained with specific antibodies (open histograms) or isotype controls (filled histograms). (c) Frequency of c-kit<sup>+</sup> Sca-1<sup>−</sup> B220<sup>+</sup> PDCA-1<sup>−</sup> IgM<sup>−</sup> cells emerging among BM cells after 18 h of incubation with different TLR agonists. CpG-B was tested in BM cell cultures of both WT and MyD88<sup>−/−</sup> C57BL/6J mice. Results are expressed as means ± s.e.m. from three experiments. *P < 0.05, **P < 0.005 when comparing stimulated and unstimulated BM cells, using non-parametric Mann–Whitney’s t-test. (d) Experimental protocol for MOG<sub>35–55</sub> EAE disease induction and intravenous progenitor cell transfer (60,000 cells per mouse) at day 12 post-immunization. (e,f) EAE clinical scores (mean ± s.e.m.) over 35 days of the indicated groups of mice. (e) n = 30 mice per group, except for PBS-ProB-recipient group in which n = 4 mice. (f) n = 10 mice per group; **P < 0.001 when comparing control mice injected with PBS and recipients of WT CpG-proBs by two-way repeated measures ANOVA test. **P < 0.005, between mice injected in vivo prepared CpG-proBs and other groups, non significant between all other groups.

Table 1 | Adoptive transfer of CpG-proB cells but not of control pro-B cells inhibits ongoing EAE.

| Group            | Mice | Mean maximum score ± s.e.m. | Day of onset ± s.e.m. | Disease incidence |
|------------------|------|-----------------------------|------------------------|-------------------|
| Control          | 30   | 3.51 ± 0.89                 | 13.56 ± 1.40           | 100%              |
| WT CpG-proBs     | 30   | 1.56 ± 1.26**               | 14.50 ± 2.22           | 76.7%*            |
| Control ProBs    | 20   | 3.57 ± 0.92                 | 13.30 ± 2.22           | 100%              |
| PBS-proBs        | 4    | 3.50 ± 0.05                 | 12.00 ± 0.00           | 100%              |

Clinical parameters from data in Fig. 1e, including the maximum clinical score (mean ± s.e.m.) of each treatment group and the day of disease onset (mean ± s.e.m.) among mice with EAE as well as the disease incidence over the entire observation period (35 days). Control mice received PBS injection instead of progenitor injection. Significant differences between CpG-proB recipients versus control mice are indicated: *P < 0.05, **P < 0.005, assessed by non-parametric Mann–Whitney’s Test.
CD24 and CD43, from fresh non-stimulated BM, as c-kit+Scal- B220+CD24hiCD43hi cells (Fig. 1e and Supplementary Fig. 3b), nor cells detected within BM culture with PBS over 18 h exhibiting the same phenotype as CpG-proB cells but at a 6–10-fold less frequency, termed PBS-proBs, had any effect in recipient mice (Fig. 1e). Therefore transient in vitro and in vivo TLR-9-dependent activation within the BM confers protective properties onto proB cells against ongoing EAE. A dose–response study (Supplementary Fig. 4) showed that injection of 60,000 CpG-proBs was optimal for protection against EAE. Neither 25,000 nor 90,000 injected cells provided significant protection, the latter possibly resulting from cell aggregation or, alternatively, from hindrance in access of cells into the hematopoietic niche of non-irradiated recipients.

**CpG-proB cells modify immune response and distribution.** To characterize the protective effect of the injected progenitors on encephalitogenic T cells, we analysed the expression of pro-inflammatory cytokines by CD4+ T cells in treated mice at the peak of the disease (d18–d21). In spinal cord, which is a major target organ in EAE, mice treated with CpG-proB cells had reduced numbers of CD4+ T cells producing all pathogenic cytokines tested, namely GM-CSF, IL-17 and IFN-γ (Fig. 2a,c). In LN, production of GM-CSF by CD4+ T cells was reduced, whereas expression of IL-17 and IFN-γ were not affected in treated mice compared with controls (Fig. 2b,c). In addition, production of the anti-inflammatory cytokine IL-10 by CD4+ T and B220+ B cells was enhanced in treated mice in both tissues (Fig. 2d–g), which was paralleled by an increased frequency of CD4+Foxp3+ regulatory T cells (Fig. 2h,i), showing a modestly enhanced Foxp3 expression (Fig. 2j).

To further delineate how the transferred CpG-proBs protected from EAE, we analysed immune cell distribution in recipient mice, in comparison with control mice with EAE. Remarkably, mice treated with CpG-proB cells displayed increased cell numbers and particularly CD4+ T cells in LN, while having markedly fewer total and CD4+ T cells in spinal cord compared with controls (Fig. 2k).

In sum, these data show that the administered B cells impact on the disease process both at the level of the CNS and LN.

**CpG-proB cells migrate and differentiate into mature Bregs.** To trace the migration and follow the differentiation of the administered CpG-proB immature cells, we injected 60,000 CpG-proB cells prepared from BM of congenic CD45.1 mice into CD45.2 recipient mice at day 12 after EAE induction (Fig. 3a). On day 9 after transfer, CD45.1+ cells were mainly found in reactive, cervical, axillary and inguinal, but not mesenteric, lymph nodes (LN; 113,900 ± 3,500 cells, mean ± s.e.m.), and spinal cord (27,880 ± 3,218 cells), with only few cells present in spleen (5,000 ± 1,998 cells; Fig. 3b). It is noteworthy that the total number of donor-derived cells was about threefold higher in recipients than the number of injected cells, indicating that they expanded in vivo. Using different B-cell and other lineage markers, we assessed that the transferred CpG-proBs differentiated exclusively into the B-cell lineage (Fig. 3c,d). In LN, the recovered CD45.1+ cells displayed a B220+CD19+IgM+IgD+IgG-CD14+CD21+CD23hi phenotype typical of transitional B cells (Fig. 3c). This population was, however, heterogeneous, comprising about 70% of CD11b+CD11c−, and 20% of CD5+ cells (Fig. 3c). In spinal cord, dorsal-derived B cells were comparable to their LN counterparts in their B220+CD19+IgM+IgD+IgG- phenotype (Fig. 3d) but with lower levels of CD21 and CD23 than in LN and exclusively CD11d+CD5+CD11b+, therefore similar to B10 cells. While the CD45.1+ B-cell progeny of injected progenitors represented only 0.77 ± 0.14% of B cells in the LN, they represented nearly eightfold more, that is, 5.97 ± 1.32% of B cells, in the spinal cord (Fig. 3e,f). Remarkably, CD45.1+ B cells produced the anti-inflammatory cytokine IL-10 in spinal cord, but not in LN (Fig. 3g).

These data demonstrate that the transferred CpG-proB cells give rise to more mature B cells that accumulate in reactive—cervical and inguinal—LN and inflamed CNS during EAE.

To assess whether such differentiation of CpG-proB cells into more mature B cells was required for protection against EAE, CpG-proB cells from CD45.1+Rag2−/− mice (prepared as in Supplementary Fig. 5) whose differentiation into mature B lymphocytes is blocked, were adoptively transferred. They had no effect on the disease course (Fig. 4a). Injected cells from CD45.1+Rag2−/− donors could not be found in LN or spinal cord at 3, 5 or 7 days after injection, in contrast to CpG-proB cells from WT donors, suggesting that their reduced lifespan as progenitors hampered them to confer protection against EAE. Therefore, transient treatment of BM cells with TLR-9 agonist generates proB cells that differentiate into more mature B cells with protective function in EAE. Remarkably, CpG-proB cells from Rag2−/− mice had no effect on cell numbers in LN and CNS (Fig. 4b), suggesting that this alteration of cell distribution directly contributed to the beneficial effect afforded by CpG-proB cells.

**Roles of IL-10 production by donor-derived B cells.** Since IL-10 is generally a key mediator of B-cell-mediated regulation, we assessed its importance for the suppressive function of CpG-proB cells. IL-10 was necessary for the protective function of CpG-proB cells, because mice treated with IL-10-deficient CpG-proBs (prepared as in Supplementary Fig. 5) were not protected against disease (Fig. 4c), and production of pathogenic cytokines by CD4+ T cells in spinal cord was not decreased (Fig. 4d,e). However, IL-10-deficient CpG-proBs still promoted CD4+ T cell accumulation in the reactive LN and reduced their numbers in spinal cord (Fig. 4f) suggesting the involvement of an additional mechanism in this latter effect.

Altogether, these data show that an efficient control of disease by CpG-proBs requires IL-10 production by their mature B-cell progeny. IL-10 produced by these cells is important to reduce inflammation locally in spinal cord, while trapping of immune cells in reactive LN is achieved via an IL-10-independent mechanism.

**Role of IFN-γ in LN entrapment of T cells by CpG-proBs.** To identify the IL-10-independent mechanism involved in the accumulation of immune cells in the reactive LN of CpG-proB cells-treated mice, we analysed cytokine production by these cells (Fig. 5a,b) in a comprehensive manner, because B cells primarily inhibit immunity through cytokine production27,10. Remarkably, freshly prepared CpG-proB cells did not produce IL-10 (Fig. 5a,b) or IL-35 (Fig. 5b), but produced abundant amounts of IFN-γ, with levels about 30 times higher than those detected for the other cytokines, following stimulation for 5 h with PMA plus ionomycin (Fig. 5a). Expression of IFN-γ by these cells was also confirmed by flow cytometry (Fig. 5b) and was not significantly different in WT and IL-10-deficient CpG-proBs (Supplementary Fig. 6). Moreover, the WT CD45.1+ B-cell progeny recovered from reactive LN and from the spinal cord still produced IFN-γ, although less in spinal cord than in LN, as measured by qRT–PCR analysis (Fig. 5c) and intracellular flow cytometry (Supplementary Fig. 7). Since IFN-γ has protective functions in EAE27,28, this prompted us to investigate whether IFN-γ played a role in the protective function of CpG-proB cells against EAE.
We tested the role of IFN-γ using CpG-proBs prepared from IFN-γ-deficient mice. IFN-γ-deficient CpG-proB cells did not protect recipient mice against EAE on adoptive transfer (Fig. 5d). Moreover, although the cells derived from IFN-γ-deficient CpG-proBs actually reached the spinal cord, where they released as much IL-10 as the WT progeny (Supplementary Fig. 7), they also produced there fivefold more GM-CSF than the WT progeny. They failed to reduce the encephalitogenic cytokines production by CD4+ T cells and to enhance IL-10-producing CD4+ T cells in spinal cord (Supplementary Fig. 8). Similarly, they failed to drive an accumulation of CD4+ T cells in reactive LN of recipient mice, in contrast to WT CpG-proBs (Fig. 5e). This suggested to us that IFN-γ produced by donor-derived B cells contributed to protection from disease not only by limiting encephalitogenic cytokine production within the spinal cord, but also by retaining encephalitogenic T cells in reactive LN, thereby preventing their accumulation in the CNS.

To characterize how IFN-γ produced by donor-derived B cells controlled T-cell accumulation in reactive LN, we examined its effect on expression by T cells of the chemokine receptor CCR7 known to control T-cell localization within LN39, and to be implicated in neuro-inflammation.30,31 CCR7 expression was reduced in LN CD4+ and CD8+ T cells of the CpG-proB-treated group relative to controls (Fig. 5f,g). Remarkably, CpG-proBs derived from IFN-γ-deficient mice, barely affected CCR7 staining on CD4+ T cells (Fig. 5f,g), contrasting with those derived from IL-10-deficient mice that were as efficient as WT CpG-proBs at retaining CD4+ T cells within LNs (Supplementary Fig. 9). When we performed a short acid wash (0.2 M acetic acid, 0.5 mM NaCl), known to eliminate bound cytokines, the peak of the disease. *P<0.05, **P<0.005, when comparing CpG-proB-injected mice and other groups. n = 6 mice per group (Students’ t-test).

Figure 2 | Protective effects of CpG-proBs at the peak of disease. (a–c) Frequency at day 21 of disease of CD4+ cells producing IL-17, GM-CSF, IFN-γ in spinal cord in a representative experiment (a) and in four experiments (c), and in reactive (cervical, axillary and inguinal) LN (b,c) prepared as described in the ´Methods´ section. (d–g) Production of IL-10 by CD4+ T cells and B220+ B cells from spinal cord (d,e) or reactive LN (fg) of control or CpG-proB-recipient mice in a representative experiment (d,g) and in three experiments (e,g) in which values are expressed as mean ± s.e.m. (h) Frequency within gated CD4+ cells of cells with nuclear expression of the transcription factor Foxp3 in spinal cord and reactive LN. (i) Percentages of Foxp3+ and Foxp3− CD4+ CD25+ T cells from spinal cord and reactive LN out of four experiments (i), also shown in j from a representative flow cytometry experiment in control mice (black line) and CpG-proB recipients (red line). Cells were stained for Foxp3 (open histograms) versus isotype control (filled histograms). (k) Total cell counts and percentage of CD4+ T cells within spinal cord and reactive LN of control or CpG-proB-recipient mice at the peak of the disease. *P<0.05, **P<0.005, when comparing CpG-proB-injected mice and other groups. n = 6 mice per group (Students’ t-test).
as shown by Britschgi et al.33. Therefore, the IFN-γ-dependent release of CCR7 ligands by CpG-proBs may play a role in inducing the retention of T cells in reactive LN in recipient mice.

Role of CCL19 in T-cell entrapment and disease protection

The protection afforded by B-cell-derived IFN-γ identifies a new mechanism of B-cell-mediated regulation in EAE. We sought to gain further insight into this mechanism. Given that IFN-γ can modulate chemokine expression27, we evaluated whether CpG-proB cells and/or their progeny themselves might be a source of CCL19 and/or CCL21 via a mechanism controlled by IFN-γ. To this end, we first quantified CCL19 and CCL21 mRNA in WT CpG-proBs, IFN-γ-deficient CpG-proBs, and IFN-γ-deficient CpG-proBs cultured in presence of IFN-γ. We found that WT CpG-proB cells produced both CCL19 and CCL21 (Fig. 6a). The production of these chemokines was reduced in IFN-γ-deficient CpG-proBs, and this defect could be erased by exogenous addition of IFN-γ (Fig. 6a). Remarkably, in LN, the mature B-cell progeny generated from CpG-proBs in recipients expressed markedly higher levels of CCL21, and even more CCL19 than they do in spinal cord and both expressions were higher than in the originally administered CpG-proB cells (Fig. 6b). CCL19 has been shown to induce CCR7 internalization more efficiently than CCL21 (ref. 33). In addition, CD4+ and CD8+ LN T cells from mice that received WT but not Ccl19-deficient CpG-proBs displayed reduced cell surface expression of CCR7 (Fig. 6c,d).

Moreover, CpG-proBs sorted from Ccl19-deficient mice neither restrained immune cells in reactive LN (Fig. 6e), nor provided protection against EAE (Fig. 6f), confirming that CCL19 production by CpG-proB cells was essential for T-cell sequestration inside the LN and protection against disease. From these data, we conclude that CCL19 produced by CpG-proB-derived B
cells in response to autocrine IFN-γ induces sequestration of T cells via CCR7 in the reactive LN, subsequently limiting their accumulation in the CNS and improving the disease course.

Discussion
In adults, immature B cells develop in BM and then seed secondary lymphoid organs, where they can acquire diverse effector functions depending on their mode of activation. Here, we show that signals perceived by immature B cells in a BCR-independent manner can determine their subsequent effector functions depending on their mode of activation. Here, we show that signals perceived by immature B cells in a BCR-independent manner can determine their subsequent effector functions depending on their mode of activation. Here, we show that signals perceived by immature B cells in a BCR-independent manner can determine their subsequent effector functions depending on their mode of activation.

The latter was achieved through LN retention of T cells, which limiting local inflammation in CNS through IL-10 production,mediated this protection because the latter depended on environment in recipient mice. Donor-derived mature B cells effect even though they encountered the same inflammatory environment in recipient mice.

Donor-derived mature B cells recovered from BM cultures shortly stimulated with CpG-B or from the BM of CpG-injected mice markedly reduced the severity of EAE in recipient mice, while unstimulated pro-B cells had no effect even though they encountered the same inflammatory environment in recipient mice. Donor-derived mature B cells mediated this protection because the latter depended on RAG expression.

The protection from disease mediated by the transferred CpG-proB cells involved two complementary mechanisms, one limiting local inflammation in CNS through IL-10 production, and the other restraining encephalitogenic T cells in reactive LN. The latter was achieved through LN retention of T cells, which was mediated by increased CCL19 production by CpG-proB-derived B cells under IFN-γ autocrine control. None of these effects were observed with control unstimulated pro-B cells.
These data demonstrate that transient changes in the BM environment of pro-B cells can markedly influence their subsequent effector functions as mature B cells in periphery, favouring their anti-inflammatory activities.

The production of IL-10 is a key mechanism, by which B cells can suppress autoimmune diseases\(^4\)–\(^6\),\(^11\). CpG-proBs did not produce IL-10 themselves, but their mature progeny became IL-10-competent strictly in spinal cord of recipient mice. Although CpG-proBs from IL-10-deficient mice normally retained immune cells in reactive LN, this was insufficient to provide protection against EAE since at the onset of clinical signs, pathogenic cells have already reached the CNS and triggered neuro-inflammation. We propose that IL-10 produced by donor-derived B cells is particularly important in the CNS in our model. In addition, the prevalence of IL-10 over GM-CSF production in the spinal cord by the progeny of CpG-proBs may be essential to control neuro-inflammation, as shown in Supplementary Fig. 7. The enhanced production of IL-10 by host CD4\(^+\) T cells and B220\(^+\) B cells in CNS of mice treated with WT CpG-proB might further help to control local inflammation in the target organ. In addition, mice treated with WT CpG-proB cells displayed an increased expansion of Tregs, suggesting that
host Tregs are also involved in the protective effect of the transferred B cells, in line with results from other studies. An important finding of this study is the identification of a novel mechanism of B-cell-mediated immune regulation involving IFN-γ production by B cells. Looking for other non-IL-10 possible mediators for the protective effects of CpG-proBs against EAE, we found that both CpG-proBs and their LN but not spinal cord progeny displayed a massive production of IFN-γ that subsequently polarize T cells towards a type 1 immune response. Bao et al. recently reported that innate B cells with a CD11a<sup>hi</sup> CD16/32<sup>hi</sup> phenotype that emerged from follicular B cells on bacterial and viral pathogen-infected animals give rise to so-called Be1 effector B cells at low levels, whereas mature follicular and marginal zone B cells produce massive amounts of IFN-γ. Co-culture with Th1 cells emerging in TLR-activation that condition the Th1 response to Salmonella enterica infection. Co-culture with Th1 cells emerging in the progeny of B cells at different levels of maturation and in various activation conditions. Thus BM immature B cells constitutively produce IFN-γ at low levels, whereas mature follicular and marginal zone B cells produce massive amounts of IFN-γ on TLR-activation that condition the Th1 response to Salmonella enterica infection. Co-culture with Th1 cells emerging in pathogen-infected animals give rise to so-called Be1 effector B cells producing large amounts of IFN-γ that subsequently polarize T cells towards a type 1 immune response. Bao et al. recently reported that innate B cells with a CD11a<sup>hi</sup> CD16/32<sup>hi</sup> phenotype that emerged from follicular B cells on bacterial and viral

Figure 6 | Role of CCL19 in the protective properties of CpG-proBs. (a) CCL19 and CCL21 mRNA levels relative to HPRT1 in WT or IFN-γ<sup>−/−</sup> CpG-proB cells isolated from BM cells stimulated with CpG-B or CpG-B + IFN-γ for 18 h followed by PMA + ionomycin for 5 h. Data are expressed as means ± s.e.m. of three experiments. *P < 0.05 by the non-parametric Mann-Whitney’s t-test. (b) CCL19 and CCL21 mRNA levels were assessed as in a in the progeny of WT CD45.1<sup>+</sup> CpG-proBs recovered in reactive LN and spinal cord of CD45.2<sup>−</sup> recipients 6 days after adoptive transfer of CD45.1<sup>−</sup> CpG-proBs, and compared with levels in WT CpG-proBs. Data are expressed as mean ± s.e.m. from 3–5 experiments. *P < 0.05 (Students’ t-test). (c,d) CCR7 expression analysis in CD4<sup>+</sup> and CD8<sup>+</sup> T cells from reactive LN of control mice (black line) and mice transferred with WT-CpG-proBs (red line) or CCL19-KO CpG-proBs (blue line), after staining with a specific anti-CCR7 antibody (open histograms) or with an isotype control (filled histogram) of intact and permeabilized cells. A representative experiment is shown in a and the mean ± s.e.m. out of three experiments in d. (e) Control mice or mice injected at day 12 with CpG-proBs derived from WT or CCL19-deficient donors were killed on day 21 of the disease and reactive LN and spinal cord cells counted. Shown are total cell counts and percentage of CD4<sup>+</sup> T cells recovered per organ, *P < 0.05, **P < 0.005 (Students’ t-test). (f) EAE clinical scores (mean ± s.e.m.) in control mice (n = 10) or mice injected either with WT (n = 10) or CCL19-deficient CpG-proBs (n = 10). ***P < 0.001, between controls or CCL19<sup>−/−</sup> and WT CpG-proB recipients, non significant between controls and CCL19<sup>−/−</sup> CpG-proB-treated mice, using the two-way repeated measures ANOVA test.
CpG-proB’s progeny releases IL-10 (5), that reduces IL-17 and GM-CSF production by T cells, enhances Foxp3 T cells via CCR7 (3), leading to effector T cells (Teff) accumulation in reactive LN. As a result, fewer T cells reach the spinal cord (4). In the spinal cord, the release by host B cells.

In untreated mice, autoreactive T cells migrate to the spinal cord (1) and release pathogenic cytokines IL-17 and GM-CSF (2). Ongoing EAE + progenitors Ongoing EAE

Conversely, Olalekan et al. reported that IFN-γ production by B cells was essential for the development of autoimmune experimental arthritis. Therefore, IFN-γ production by CpG-proBs as well as other described B cells have functional, either protective or deleterious, roles and may link innate and adaptive immune responses.

Since PD-L1hi mature splenic B cells were recently shown to provide protection against EAE and PD-L1 can be induced by IFN-γ, we investigated the possible role of PD-L1 in CpG-proB-induced protection. There was no correlation between the level of PD-L1 expression on WT versus IL-10 and IFN-γ-deficient CpG-proBs and their relative protective capacity against EAE (Supplementary Fig. 10a). Furthermore, CpG-proBs isolated from PD-L1-deficient C57BL/6 donor mice showed only a slight (non significant) reduction in their protective effect against EAE (Supplementary Fig. 10b). Therefore, PD-L1 is not playing a significant role in disease protection against EAE by CpG-proBs.

Our data indicate that the protective effect mediated by B-cell-derived IFN-γ operates at least in part by sequestering CD4+ T cells in the reactive LN. Many presently available treatments of MS aim at preventing T cells from entering the CNS. Several chemokine receptors and their ligands have been shown to take part in the process of neuroinflammation, including CCR7 whose ligands CCL19 and CCL21 are produced by endothelial cells at the inflamed BBB in mice and men. CCR7hi mature splenic B cells were recently shown to take part in the process of neuroinflammation, including CCR7 whose ligands CCL19 and CCL21 are produced by endothelial cells at the inflamed BBB in mice and men. Consequently, Olalekan et al. reported that IFN-γ production by B cells was essential for the development of autoimmune experimental arthritis. Therefore, IFN-γ production by CpG-proBs as well as other described B cells have functional, either protective or deleterious, roles and may link innate and adaptive immune responses.

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Figure 7 | Protection against EAE by CpG-proBs relies on cooperative action of their LN and spinal cord B-cell progeny. (a) At the onset of clinical signs of EAE in untreated mice, autoreactive T cells migrate to the spinal cord (1) and release pathogenic cytokines IL-17 and GM-CSF (2). (b) In mice protected against EAE by adoptive transfer of only 60,000 CpG-proBs per recipient, the IFN-γ-dependent release by CpG-proB’s progeny of CCL19 (1–2) anchors T cells via CCR7 (3), leading to effector T cells (Teff) accumulation in reactive LN. As a result, fewer T cells reach the spinal cord (4). In the spinal cord, the CpG-proB’s progeny releases IL-10 (5), that reduces IL-17 and GM-CSF production by T cells, enhances Foxp3 T cells, and impedes leucocyte migration. The particular anchoring role of CCL19 produced by the B-cell progeny was confirmed by the findings that CpG-proB cells isolated from CCL19-deficient mice did not reduce surface CCR7 expression measured without acidic washing procedure and concomitantly.
lost their capacity to sequester T cells within reactive LN, and to protect against EAE disease. Importantly, the markedly (3,000-fold) enhanced production of CCL19 in LN rather than spinal cord progeny of CpG-proBs strongly supports a major role of this protective mechanism in the reactive LN to keep T cells away from the CNS. Notably, we did not observe significant reduction of S1P1R expression of LN-trapped T cells in CpG-proB recipients relative to control mice with EAE. Therefore, different from FTY720 (ref. 48) which targets the LN egress signal, CpG-proBs promote entrapment of pathogenic T cells inside secondary lymphoid organs by targeting instead the selective molecular mediators that may be necessary to provide full demonstration of their roles in the protection afforded by CpG-proBs. CpG-proBs are, to our knowledge, the most efficient B-cell subset that can be exploited to stably inhibit ongoing encephalomyelitis. These properties of CpG-proBs therefore open interesting perspectives for cell therapy of autoimmune diseases. In addition, these data shed light on the importance of the BCR-independent signals that B-cell progenitors receive in the BM environment to develop into mature B cells with regulatory rather than inflammatory functions.

Methods

Mice. WT CD45.2- C57BL/6 mice were obtained from Janvier Laboratories (Le Genest Saint Isle, France). Congenic CD45.1-MyD88-/-, IFN-γ-/-, Rag2-/- and C57BL/6 mice were provided by Dr. Stevanović at Trinity Biomedical Sciences Institute, Trinity College Dublin. Knock-out animals and their WT controls from the same origin were systematically used as donor mice for CpG-proBs. Female 12-week-old C57BL/6 mice were used as a model for MOG35-51-induced EAE and received intravenous progenitor cell transfers at day-12 post-immunization.

EAE induction. Active EAE was induced in 12-week-old female mice by s.c. immunization at two sites, upper and lower back, with 200 μg MOG35-51 peptide emulsified in CFA containing 400 μg heat-killed Mycobacterium tuberculosis H37Ra (Hooke Laboratories, Lawrence, MA, USA), on day 0. In addition, mice received 200 ng pertussis toxin (Hooke Laboratories) i.p. in 0.1 ml per mouse on days 0 and 1. Clinical signs of EAE were assessed daily with a 0–4 scoring system defined as follows: 0, no obvious changes in motor function compared with the non-immunized mice; 0.5, tip of tail is limp; 1, limp tail; 1.5, limp tail and hind leg inhibition; 2, limp tail and weakness of hind legs; 2.5, limp tail and dragging of hind legs; 3, limp tail and complete paralysis of hind legs or paralysis of one front and one hind leg; 3.5, limp tail and complete paralysis of hind legs that are together on one side of body; 4, limp tail, complete hind leg and partial front leg paralysis; mouse is still markedly moving and appears feeding: 4.5, complete hind leg and partial front leg paralysis; no movement around the cage, mouse is not alert; 5, death or severe paralysis. Mice with score ≥4 for two consecutive days and mice with score 5 were killed.

Sorting of TLR-activated BM progenitors. BM cells removed from tibiae and femurs of 8–12-week-old C57BL/6 mice were incubated in RPMI-1640 medium (PAA) supplemented with 10% (vol/vol) fetal calf serum and 1% antibiotics (penicillin and streptomycin) for 18 h with 1 μg ml-1 of the oligodeoxynucleotides CpG 1,686 (CpG-A: InvivoGen), CpG 1,585 (CpG-B; Eurogentec, Angers, France) or with the respective agonists of TLR1–9, supplied in a commercial kit (InvivoGen, Toulouse, France), including: Pam3CSK4 (0.5 μg ml-1), HKLM (2 × 106 cells ml-1), Poly I:C (100 μg ml-1), Poly I:C (5 μg ml-1), LPS-EP standard (1 μg ml-1), FLA-31 standard (1 μg ml-1) and ssRNA40/Lo/Vec (2 μg ml-1) as well as CpG L1,826 (CpG-B; 1 μg ml-1).

C-kit+ BM cells were sorted by immuno-magnetic separation using a RoboSep automation (StemCell Technologies, Grenoble, France). Sorted cells were further stained with appropriate fluorochrome-conjugated mAbs and analyzed using a FACSAria I (BD Biosciences, Le Pont de Claix, France).

Isolation of immune cells from the spinal cord. Spinal cords isolated from control and CpG-proB-recipient mice were incubated for 30 min in digestion buffer of DNase and liberase (10 mg ml-1 in PBS) at 37 °C, mixing every 5 min. EDTA (100 mM, 500 μl) was added for 1 min to end the digestion. The cells were passed through a cell strainer, using a syringe plunger (back side) to break the tissues. Cells were then resuspended in 3–5 ml of 40% Percoll underlayered with the same volume of 70% Percoll (in PBS) and centrifuged for 35 min at 1,300 g (2,800 r.p.m.) without brakes to form a smooth interface. Cells were collected with a Pasteur pipette and diluted 10 times with complete medium RPMI 10% SFV. After centrifugation cells were resuspended in 2–3 ml of complete medium and further stained with appropriately labelled mAbs.

Staining of cells for flow cytometry analysis. To block non-specific Fc receptor binding, cells were pre-incubated for 10 min at room temperature with FcR blocker 2.4G2 mAb. Cells were then stained with appropriately labelled mAbs against CD45, CD25, CD44, CD45R0, CD11b, PD-L1, CD14, CD16, CD19, CD27, CD25, CD11b, c-Kit, PDCA-1, C8d (eBioscience, Paris, France), CD19, C27, CD25, CD45.1 (BD Biosciences) and PDCA-1 (Miltenyi Biotec, Paris, France). Nuclear Foxp3 expression was measured by FACS analysis as per the manufacturer’s instructions (eBioscience). Pax5 expression was measured in B-cell progenitors permeabilized with the same buffer as for Foxp3, using an anti-Pax5 antibody from eBioscience. Intracytoplasmic expression of cytokines was assessed after a 5-h stimulation with PMA (10 ng ml-1) plus ionomycin (500 ng ml-1) in the presence of Brefeldin A (20 μg ml-1), followed by fixation/permeabilization with PFA/saponin and subsequent staining with specific antibodies including APC-labelled anti-IL-23p19, APC-labelled anti-IL-17, PE-labelled anti–IFN-γ, APC-labelled anti-IL-17 (all from BD Biosciences), PE-labelled anti-GM-CSF (from eBioscience), Per-CP-labelled anti-human/mouse IL-12/IL-35 p35 (from RnD Systems) or isotype controls (from BD Biosciences and eBioscience).

Membrane and intracellular antigen expression was analysed in a FACS Canto II cytometer (BD Biosciences) using FlowJo software (Treestar, Ashland, OR, USA).

Cytokine assays. Control and CpG-ProB-recipient mice were killed at the peak of the disease, 18–21 days after immunization. Cytokines were measured in supernatants of recovered LN cells from control or CpG-ProB-recipient mice, re-stimulated with the MOG35–51 peptide for 3 days followed by 5-h stimulation with PMA + ionomycin. For determination of spinal cord cytokines, isolation of spinal cord cells was performed by incubation at 37 °C/5% CO2 in the presence of GMP + FMA (eBioscience) for 3 h, Th1/Th2/Th17 cytokines anti-GM-CSF were measured by multiplex ELISA using Flow Cytomix analyte detection reagents from eBioscience.

Isolation of mRNA and real-time qRT-PCR. Total RNA was prepared using RNeasy-4PCR (Ambion, Life Technologies, St Aubin, France). Reverse transcription was performed with high-capacity cDNA reverse-transcription kits (Applied Biosystems, Life Technologies). Resulting cDNA was amplified in triplicates by the SYBR-Green PCR assay. PCR reactions were incubated for 2 min at 50 °C and for 10 min at 95 °C, followed by 40 amplification cycles with 1 min annealing/extension at 60 °C and 15 s denaturation at 95 °C. Quantitative real-time PCR of mouse CCL19 and CCL21 was performed by the comparative threshold cycle (ΔΔC) method and normalized to human HBB as an internal control. Primer sequences were used for CCL19 to identify functional CCL19 (ref. 50). Primer sequences were as described: CCL19 forward: 5′-GGTGCTCAGGATATTAGCCGAT-3′, CCL19 reverse: 5′-GGTCCTTCCACTTACAGGAC-3′; CCL21 forward: 5′-ATCCCGGCACACTGTGTTCTC-3′, CCL21 reverse: 5′-GGTTCTCGCAACCCAGGCTT-3′; HPRT1 forward: 5′-CCTTCTACCAATGACTCTAGTAC-3′, HPRT1 reverse: 5′-AAAGTTTACAGCAGAAGTC-3′.

Study approval. All mouse procedures were approved by the Paris Descartes University Animal Experimentation and Ethics Committee. Sample sizes were chosen to ensure reproducibility of the experiments and according to the 3Rs of animal ethics regulation.
Numerous studies have demonstrated that regulatory B cells (B10 cells) expressing interleukin-10 (IL-10) play a crucial role in the suppression of autoimmunity. These cells regulate autoimmunity by providing IL-10, which inhibits the activation and function of effector T cells and promotes the differentiation of regulatory T cells (Tregs) [1].

IL-10-producing B cells have been shown to regulate autoimmune effector T cell activation in various models of chronic autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) [2]. These cells suppress the development and function of effector T cells by producing IL-10, which inhibits the production of pro-inflammatory cytokines and chemokines [3].

In addition to IL-10, B10 cells also produce transforming growth factor-beta (TGF-β), which plays a critical role in the suppression of autoimmune responses [4]. TGF-β is known to inhibit the proliferation and differentiation of effector T cells, as well as the production of inflammatory cytokines [5].

B10 cells are also known to express Fas ligand (FasL), which can induce apoptosis in effector T cells upon engagement with Fas-expressing T cells [6]. FasL-Fas interactions have been implicated in the suppression of autoimmune responses in various experimental models [7].

Furthermore, B10 cells can interact with Tregs through the expression of CD40, a co-stimulatory molecule that promotes Treg proliferation and function [8]. This interaction enhances the suppressive activity of Tregs and further contributes to the regulation of autoimmune responses [9].

Collectively, these findings highlight the critical role of B10 cells in the suppression of autoimmune responses. Their ability to produce IL-10, TGF-β, and FasL, as well as their capacity to interact with Tregs through CD40, underscores their potential as therapeutic targets for the treatment of autoimmune diseases [10].

References
1. Lehmann-Horn, K., Kronsbein, H. C. & Weber, M. S. Targeting B cells in the treatment of autoimmune diabetes. Nat. Rev. Immunol. 3, 944–950 (2003).
2. Mizoguchi, A., Mizoguchi, E., Takedatsu, H., Blumberg, R. S. & Bhan, A. K. Annu. Rev. Immunol. 16, 219–230 (2002).
3. Fillatreau, S., Sweeney, C. H., McGeachy, M. J., Gray, D. & Anderton, S. M. B cells regulate autoimmunity by provision of IL-10. Nat. Immunol. 3, 944–950 (2002).
4. Mizoguchi, A., Mizoguchi, E., Takedatsu, H., Blumberg, R. S. & Bhan, A. K. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. Immunity 16, 219–230 (2002).
5. Tran, E. H., Prince, E. N. & Owens, T. IFN-gamma shapes immune invasion of the central nervous system via regulation of chemokines. J. Immunol. 164, 2759–2768 (2000).
6. Yanaba, K. et al. IFN-gamma signaling to astrocytes protects from autoimmune mediated neurological disability. PloS ONE 7, e42088 (2012).
7. Thill, T. B. et al. Targeting B cells in the treatment of multiple sclerosis: recent advances and remaining challenges. Ther. Adv. Neurol. Disord. 6, 161–173 (2013).
8. Mizoguchi, A., Mizoguchi, E., Takedatsu, H., Blumberg, R. S. & Bhan, A. K. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. Immunity 16, 219–230 (2002).
9. Fillatreau, S., Sweeney, C. H., McGeachy, M. J., Gray, D. & Anderton, S. M. B cells regulate autoimmunity by provision of IL-10. Nat. Immunol. 3, 944–950 (2002).
10. Mauri, C., Gray, D., Muiuhaq, N. & Lendem, P. Prevention of arthritis by interleukin-10-producing B cells. J. Exp. Med. 197, 489–501 (2003).
11. Evans, J. G. et al. Novel suppressive function of transitional 2 B cells in experimental arthritis. J. Immunol. 178, 7688–7807 (2007).
12. Yanaba, K. et al. Regulatory B cell subset with a unique CD1dhiCD5þ phenotype controls T cell-dependent inflammatory responses. Immunity 28, 3594–3608 (2008).
13. Shimomura, Y. et al. Regulatory role of B-1 B cells in chronic colitis. Int. Immunol. 20, 729–737 (2008).
14. Shen, P. et al. IL-35-producing B cells are critical regulators of immunity during autoimmunity and infectious diseases. Nature 507, 366–370 (2014).
15. Matsumoto, M. et al. Interleukin-10-producing plasmablasts exert regulatory function in autoimmune inflammation. Immunity 41, 1040–1051 (2014).
16. Tan, A. R. et al. IL-10-producing regulatory B cells are critical regulators of humoral immunity. Nat. Commun. 6, 5997 (2015).
17. Lampropoulou, V. et al. TLR-activated B cells suppress T cell-mediated autoimmunity. J. Immunol. 180, 4763–4773 (2008).
18. Yanaba, K., Bouaziz, J. D., Matsuhashi, T., Tsutaya, T. & Tedder, T. F. The development and function of regulatory B cells expressing IL-10 (B10 cells) requires antigen receptor diversity and TLR signals. J. Immunol. 182, 7459–7472 (2009).
19. Mauri, C. & Bosma, A. Immune regulatory function of B cells. Annu. Rev. Immunol. 30, 221–242 (2012).
20. Baba, Y., Matsumoto, M. & Kurosaki, T. Calcium signaling in B cells: regulation of cytokine production and its sensor molecules, STIM1 and STIM2. Mol. Immunol. 62, 339–343 (2014).
21. Mann, M. N., Marek, G., Shriver, L. P., Tan, Y. & Dittell, B. N. B cell regulation of CD4 þ CD25 þ regulatory T cells and IL-10 via TGF-beta is essential for recovery from experimental autoimmune encephalomyelitis. J. Immunol. 178, 3447–3456 (2007).
22. Yoshizaki, A. et al. Regulatory B cells control T-cell autoimmunity through IL-21-dependent cognate interactions. Nature 491, 264–268 (2012).
23. Blair, P. A. et al. Selective targeting of B cells with agonistic anti-CD40 is an efficacious strategy for the generation of induced regulatory T2-like B cells and for the suppression of lupus in MRL/lpr mice. J. Immunol. 182, 3492–3502 (2009).
24. Duddy, M. et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. J. Immunol. 178, 6092–6099 (2007).
25. Correale, J. & Farez, M. F. Does helminth activation of toll-like receptors arrest or promote disease? Front. Cell. Infect. Microbiol. 2, 112 (2012).
26. Ramgolam, V. S. et al. B cells as a therapeutic target for IFN-beta in relapsing-remitting multiple sclerosis. J. Immunol. 186, 4518–4526 (2011).
27. Begum-Haque, S. et al. Augmentation of regulatory B cell activity in experimental allergic encephalomyelitis by glatiramer acetate. J. Neuroimmunol. 223, 136–144 (2011).
28. Kala, M. et al. B cells from glatiramer acetate-treated mice suppress experimental autoimmune encephalomyelitis. Exp. Neurol. 221, 136–145 (2010).
29. Montandon, B. et al. Innate pro-B-cell progenitors protect against type 1 diabetes by regulating autoimmune effector T cells. Proc. Natl Acad. Sci. USA 110, 12718–12723 (2013).
30. Welner, R. S. et al. Lymphoid precursors are directed to produce dendritic cells as a result of TLR9 ligation during herpes infection. Blood 112, 3753–3761 (2008).

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Author contributions
S.K., F.Z. designed and performed the experiments, interpreted the data and wrote the manuscript; C.G., H.L., R.M. and J.M. designed and performed the experiments and analysed the data; S.S. performed the experiments; S.E., P.G.F., S.A.L. and S.F. provided essential material, as well as a critical review of the data and manuscript.

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