Regulatory T cells (T\(_R\)s) can suppress the function of other effector T cells in the setting of autoimmunity, transplantation, and resistance to tumors. The mechanism for the induction of T\(_R\)s has not been defined. We previously reported that an injection of immature dendritic cells (DCs) pulsed with influenza matrix peptide (MP) led 7 days later to antigen-specific silencing of effector T-cell function in the blood of 2 healthy human subjects. Here, we found that interferon-\(\gamma\)–producing effectors return by 6 months. Importantly, in mixing experiments, CD8\(^+\) T cells from the sample obtained 7 days after injection could suppress MP-specific effectors obtained before injection and those in recovery samples. This suppression or regulation was specific for the immunizing peptide (MP) and cell-dose dependent, and it required contact between the 2 samples. These data show the capacity of immature DCs to induce antigen-specific regulatory CD8\(^+\) T cells in humans. (Blood. 2002;100:174-177)

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**Materials and methods**

**Study design and injection of DCs**

This report describes the findings in 2 volunteer subjects (Im1 and Im2) who received subcutaneous injections of immature DCs derived by culture...
of blood monocyte precursors in granulocyte-macrophage colony-stimulating factor and IL-4 as described previously. The injected DCs were pulsed with keyhole-limpet hemocyanin and influenza MP during the last 16 hours of a 6-day (Im1) or 7-day (Im2) monocyte culture as described previously.

Follow-up and immune monitoring

Both subjects were evaluated 1 week after DC injection and at 1- to 3-month intervals afterward. Both had normal results on repeated hemogram assessments and no rheumatoid factor or antinuclear antibody 1 and 3 months after DC injection. Antigen-specific T cells were quantified by using a standard enzyme-linked immunospot (ELISPOT) assay for the presence of peptide-specific cells producing IFN-γ, IL-4, or IL-10. For cytolytic T-lymphocyte (CTL) assays, T cells were cocultured with peptide-pulsed mature DCs for a week, before measurement of lytic activity, as described previously. DC maturation was achieved by day 1 of culture in a mixture of blood mononuclear cells (PBMCs) obtained 7 days after immunization (TR sample), before immunization, or at recovery (eg, day 180) were thawed and cultured (2-3 × 10^5 cells/well) either separately or together (ratio, 1:1), in the presence of mature DCs pulsed with influenza MP-2, LMP-2, or HIV gag at a DC/PBMC ratio of 1:60. Antigen-specific, INF-γ–producing cells were quantified by an ELISPOT assay after overnight culture in the presence of DCs pulsed with influenza MP, EBV-LMP2, or HIV gag at a DC/PBMC ratio of 1:60. Data are representative of results from 2 similar experiments. One asterisk indicates P < .05 for comparison with baseline reactivity on Student t-test. The asterisk indicates P < .05 for comparison with baseline reactivity on Student t-test. (B) Dose-dependent inhibition of T-cell function. (C) Characterization of peptide-specific TR α. PBMCs (3 × 10^5 cells/well) from recovery specimens (day 180) from subject Im2 were mixed with preimmunization specimens (nonsuppressor) or various doses of specimens obtained on day 7 (suppressor; ratio of day 7 to day 180 samples, 1:1 or 1:10). Antigen-specific, INF-γ–producing cells were quantified by an ELISPOT assay after overnight culture in the presence of DCs pulsed with influenza MP, EBV-LMP2, or HIV gag at a DC/PBMC ratio of 1:60. Data are representative of results from 2 similar experiments. One asterisk indicates P < .05 for comparison with baseline reactivity on Student t-test. Two asterisks indicate P < .05 for comparison with the suppressed reactivity. Antigen-specific, INF-γ–producing cells were quantified by an ELISPOT assay after overnight culture in the presence of DCs pulsed with influenza MP, EBV-LMP2, or HIV gag at a DC/PBMC ratio of 1:60. Data are representative of results from 2 similar experiments. One asterisk indicates P < .05 for comparison with baseline reactivity on Student t-test. Two asterisks indicate P < .05 for comparison with the suppressed reactivity.
Statistical analysis
The Student t test was used to compare results in different groups. The significance level was set at P < .05.

Results
Both healthy volunteers had been primed to influenza at baseline because influenza MP–specific effector T cells were detectable on ELISPOT assays and peptide-specific CTLs could be expanded by a week of culture with mature DCs. However, 1 week after the injection of MP-pulsed immature DCs, these effector functions in the blood were silenced. This loss of function was reversible, with values returning to preinjection levels by 3 to 4 months after injection in both subjects (Figure 1). In a reciprocal fashion, silencing and recovery of effector T-cell function were associated with the appearance and then decline in peptide-specific IL-10 producers, which were no longer detectable after 90 to 100 days postimmunization (Figure 1). The DC injections were not associated with any clinical toxicity or clinical or serologic evidence of autoimmunity in either subject. Thus, the inhibition of effector T-cell function after a single injection of immature DCs was found to be self-limited.

Because we had previously shown that the loss of circulating MP-specific effector T-cell function was not associated with a decline in circulating MHC tetramer–binding cells,22 we tested whether the effector silencing after injection of immature MP-pulsed DCs was mediated by the induction of Tregs. To assess this directly, we mixed T cells from samples obtained 1 week after immunization (when the effector silencing was maximal) with samples obtained before immunization. The PBMCs obtained on day 7 inhibited MP-specific producers of INF-γ from cultures of preimmunization samples from both subjects (Figure 2A). The inhibition was specific for the immunizing peptide because responses to the control peptide LMP-2 were not silenced.

Further characterization of the suppression was carried out only in samples from Im2, from whom we had additional cells available. The suppression of T-cell function was not due simply to competition for APCs or consumption of IL-2, since it was specific for immunizing peptide and observed only when suppressor samples (day 7) but not nonsuppressor (preimmunization) samples were added to the recovery samples (day 180; Figure 2B). T-cell suppression was dose dependent and observed even when the ratio of day-7 to day-180 cells was 1:10. Suppression was lost if day-7 cells were depleted of CD8+ T cells or if cell contact between day-7 and recovery T cells was prevented in transwell cultures (Figure 2C). Although the day-7 specimens had been shown to contain MP-specific IL-10 producers, addition of neutralizing anti–IL-10 antibody led to only a slight recovery of MP-specific effectors. However, the suppression was fully reversed by addition of 100 U/mL rIL-2. Thus, we found that peptide-specific CD8+ T cells induced in vivo by immature DCs inhibit CD8+ T cells in a cell-contact–dependent manner, that is, a manner largely independent of IL-10.

Discussion
These data provide direct evidence for the existence of antigen-specific CD8+ T-cell–mediated immune regulation and of the induction of such T cells in vivo in humans by immature DCs. Once induced, these cells have a limited life span in the circulation. Thus, naturally occurring Tregs may require continued antigen presentation by trafficking immature DCs. Because peptide-specific, IL-10–producing cells are also induced by immature DCs, we refer to these suppressor cells as Tregs, in keeping with previously established nomenclature. The regulation we observed required cell-cell contact and was largely independent of IL-10. These features are similar to those of CD4+ Tregs induced by immature DCs in vitro.21 A subset of CD8+CD28- suppressor T cells that mediate suppression in a cell-contact–dependent fashion has also been described.12-14

The site where immature DCs generate Tregs in vivo is not known. One possibility is that the DCs might traffic to lymph nodes to meet T cells recirculating by means of high endothelial venules. An alternative, which we favor because Tregs have an activated phenotype, is that the DCs activate Tregs that circulate from blood to extravascular spaces (here, the skin) and then return to the lymph node by means of the lymphatics. Although our studies help to confirm the presence of Tregs and IL-10–producing cells in the CD8 compartment, compared with previously described CD4+ Tregs, further work is needed to clarify their relation to CD25+ suppressor cells and their mechanism of action.

Our data suggest DC maturation as a key therapeutic target for the regulation of immunity.19 The inhibition of maturation in antigen-capturing DCs may promote the induction of Tregs in vivo. Impairment of CD8+ T-cell suppressor function has been observed in patients with human autoimmune diseases such as lupus and multiple sclerosis.15,16 A role for Tregs in acceptance of human allografts has also been suggested.24 In a reciprocal fashion, reduction of Tregs may improve resistance to cancer and chronic infections, as was observed in a study of experimental tumors in mice.25

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References
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Antigen-bearing immature dendritic cells induce peptide-specific CD8+ regulatory T cells in vivo in humans

Madhav V. Dhodapkar and Ralph M. Steinman