

Cutting Edge: Human B Cell Function Is Regulated by Interaction with Soluble CD14: Opposite Effects on IgG1 and IgE Production¹

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The mechanism(s) controlling activation of naive B cells, their proliferation, Ag receptor affinity maturation, isotype switching, and their fate as memory or plasma cells is not fully elucidated. Here we show that between 24 and 60% of CD19⁺ cells in PBMC bind soluble CD14 (sCD14). Tonsillar B cells also bind sCD14, but preferentially the CD38^{-ve/low} cells. Interaction of sCD14 with B cells resulted in higher levels of IgG1 and marked inhibition of IgE production by activated tonsillar B cells and Ag-stimulated PBMC. We found that sCD14 interfered with CD40 signaling in B cells, inhibited IL-6 production by activated B cells, and increased the kinetics and magnitude of CD40 ligand expression on T cells. Together with the previously reported effects on T cells, these findings define sCD14 as a novel soluble regulatory factor capable of modulating cellular and humoral immune responses by interacting directly with T and B cells. *The Journal of Immunology*, 2000, 164: 3480–3485.

The mechanism(s) controlling activation of naive B cells, their proliferation, Ag receptor affinity maturation, isotype switching, and their fate as memory or plasma cells is not fully elucidated. During T cell-dependent humoral immune responses, Th cells provide critical costimulatory signals to B lymphocytes (1). Ligation of CD40 on B cells by CD40 ligand (CD40L)³ transiently expressed in Th cells is particularly important (2). CD40 activation leads to B cell proliferation, which is

supported by B cell Ag receptor (BCR) triggering and further stimulated by Th2-like cytokines, which also induce CD40-activated B cells to secrete Igs and switch isotype. Dendritic cells also contribute to the induction and progression of the humoral immune response (3, 4). Thus, the cognate T-B cell interaction together with BCR triggering and APC activity generate a regulated network of positive and negative signals that determine the fate of the B cell. Additional hitherto unidentified soluble factors, cytokines, and/or cell contact signals may be involved in this regulatory network.

CD14 plays a pivotal role in mediating cell activation induced by bacterial cell wall components (5). Soluble forms of CD14 (sCD14) are found in normal human plasma at 2–3 $\mu\text{g/ml}$ concentration (6–8), exceeding by one or two logs that of the cell membrane-bound receptor. Increased sCD14 levels were found in a number of pathological states (9–11); however, the physiological role of sCD14 has remained unclear. Recently, we demonstrated that sCD14 interacts directly (without LPS) with activated human T cells, decreasing Ag, and mitogen-induced proliferation (12). This effect results from inhibition of IL-2 production, and IFN- γ and IL-4 are similarly affected. These findings revealed a novel function of sCD14: its capacity to regulate T lymphocyte activation and function. In the course of our studies, we observed that sCD14 bound to B cells in PBMC. Here, we have studied the interaction of sCD14 with human B cells and tested the functional consequences for the T-dependent humoral immune response.

Materials and Methods

Production and purification of rsCD14

Human rsCD14 8 aa shorter at the C terminus (sCD14₍₃₄₈₎), the full-length protein (sCD14wt), alanine substitution mutants sCD14 (91–94, 96)A and sCD14 (97–101)A, prepared by two-step PCR-based mutagenesis, and biotinylated sCD14 were prepared as described (12, 13).

Cells, cultures, and flow cytometry

Tonsillar B cells were prepared from normal individuals undergoing tonsillectomy, as described (14). The purity of the B cell preparation was $\geq 97\%$ as determined by flow cytometry using anti CD19, CD14, and CD3 mAb. Tonsillar B cells, EBV⁺ B cell lines (Akata and Rael), murine L fibroblasts transfected with human CD40L (tCD40L) or CD32 (tCD32) (donated by Prof. M. Rowe, University of Wales) and PBMC from healthy donors were cultured in RPMI 1640 medium supplemented with 2 mM

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³ Abbreviations used in this paper: CD40L, CD40 ligand; BCR, B-cell Ag receptor; GC, germinal center; sCD14, soluble CD14; sCD14₍₃₄₈₎, truncated sCD14; sCD14wt, wild type sCD14; tCD40L, CD40L transfectants; tCD32, control transfectants; VZV, varicella-zoster virus.

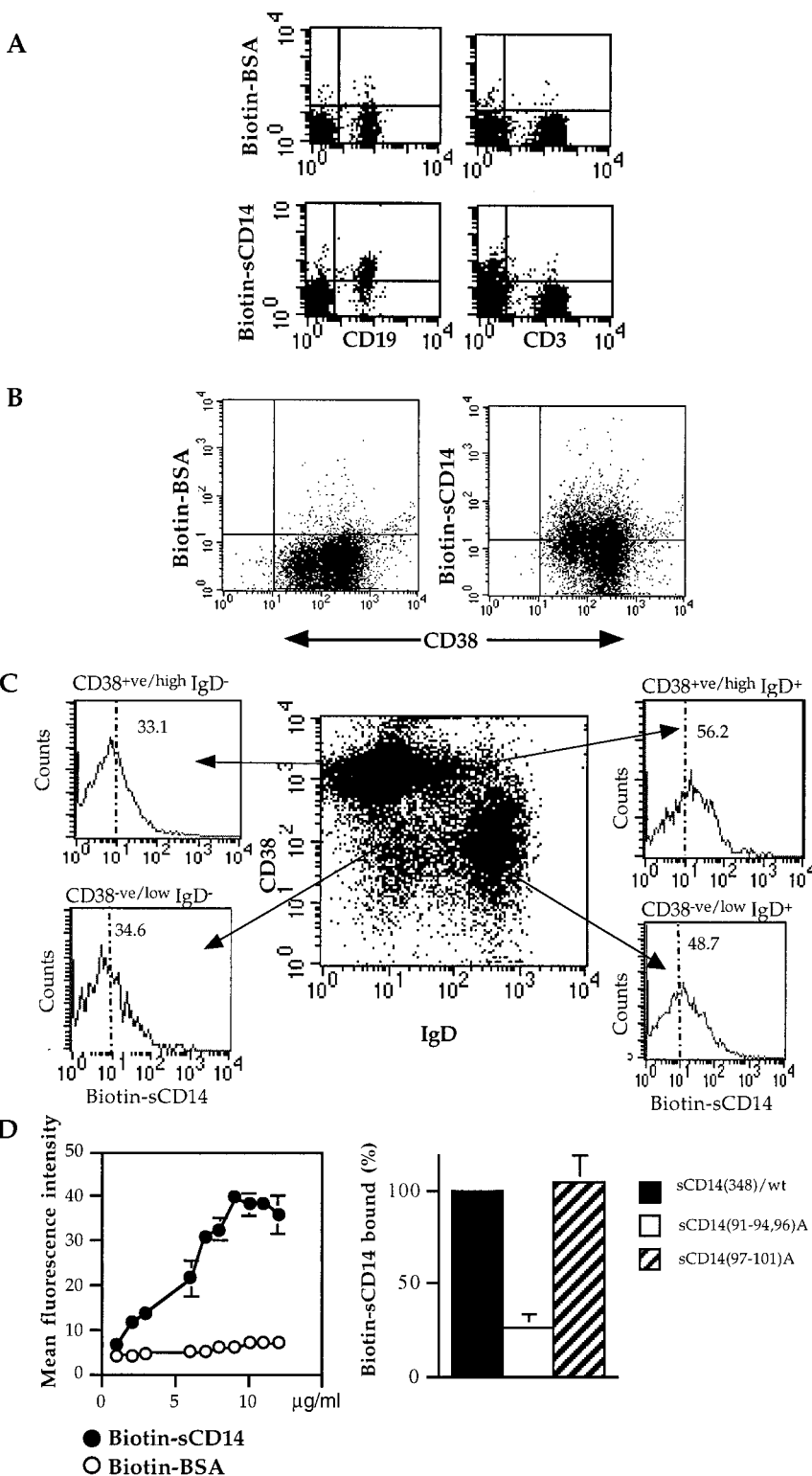


FIGURE 1. sCD14 binds to human B cells. **A**, Flow cytometric analysis of PBMC (representative of 14 donors) stained with biotinylated sCD14 or control BSA and FITC-conjugated anti-CD19 or CD3 mAb. **B**, FACS profiles representative of nine total tonsillar B cell preparations stained with sCD14 or BSA and the FITC-conjugated CD38 mAb. **C**, Tricolor FACS analysis of total tonsillar B cells stained with biotinylated sCD14 or BSA (dotted lines) followed by Tricolor-conjugated streptavidin, purified anti-CD38 mAb, followed by R-PE-conjugated rabbit anti-mouse Ab and FITC-conjugated goat anti-human IgD Ab. B cell subsets were defined by their CD38 and IgD marker expression (dot plot). Percentages of sCD14-binding cells of each B cell subset are shown. Experiments were performed three times. **D**, *left*, Saturation experiments. Tonsillar B cells (1×10^5 cells) were incubated with increasing concentrations of biotinylated sCD14 or BSA and tested for binding by flow cytometry. Results are the mean of four independent experiments. **D**, *right*, Binding capacity comparison between sCD14₍₃₄₈₎ or sCD14wt used in this study and arbitrarily set at 100%, and two alanine substitution mutants. Results are means of three experiments.

L-glutamine, 10 mM HEPES buffer, antibiotics, and 10% FCS (sCD14 from FCS was estimated to contribute about 10% to the total amount used in the experiments). For flow cytometry, cells were stained with biotinylated sCD14 or BSA as control (3 μ g/ml), the FITC-conjugated or purified anti-CD mAb indicated in *Results*, or their isotype-matched controls and analyzed with a FACScalibur cytometer (Becton Dickinson, San Jose, CA) (12).

Cell stimulation and Ig and cytokine determinations

Total tonsillar B cells (1×10^5 /well) were cultured for 10 days with 90% confluent γ -irradiated tCD40L cells (5:1, B/fibroblasts) in the presence or

absence of sCD14. Cultures were supplemented with anti-human IgM-coated beads (25 μ g/ml; anti- μ -chain, Irvine Scientific, Santa Ana, CA), IL-4 (600 IU/ml), and IL-2 (100 IU/ml) as indicated in *Results*. Culture supernatants were tested for IgG1, IgE, or IL-6 by ELISA using specific matched-paired Abs (IgG1, PharMingen, San Diego, CA; IgE, Serotec, Oxford, U.K.; IL-6, R&D Systems, Abingdon, U.K.). PBMC (2×10^5 /well) were Ag stimulated (varicella-zoster virus (VZV), Oka strain, vaccine preparation, 0.05% of a 10^4 PFU solution) in the presence or absence of sCD14. At day 2, culture supernatants were tested for IL-2 (ELISA; R&D Systems), and at day 10 were tested for IgG1 and IgE.

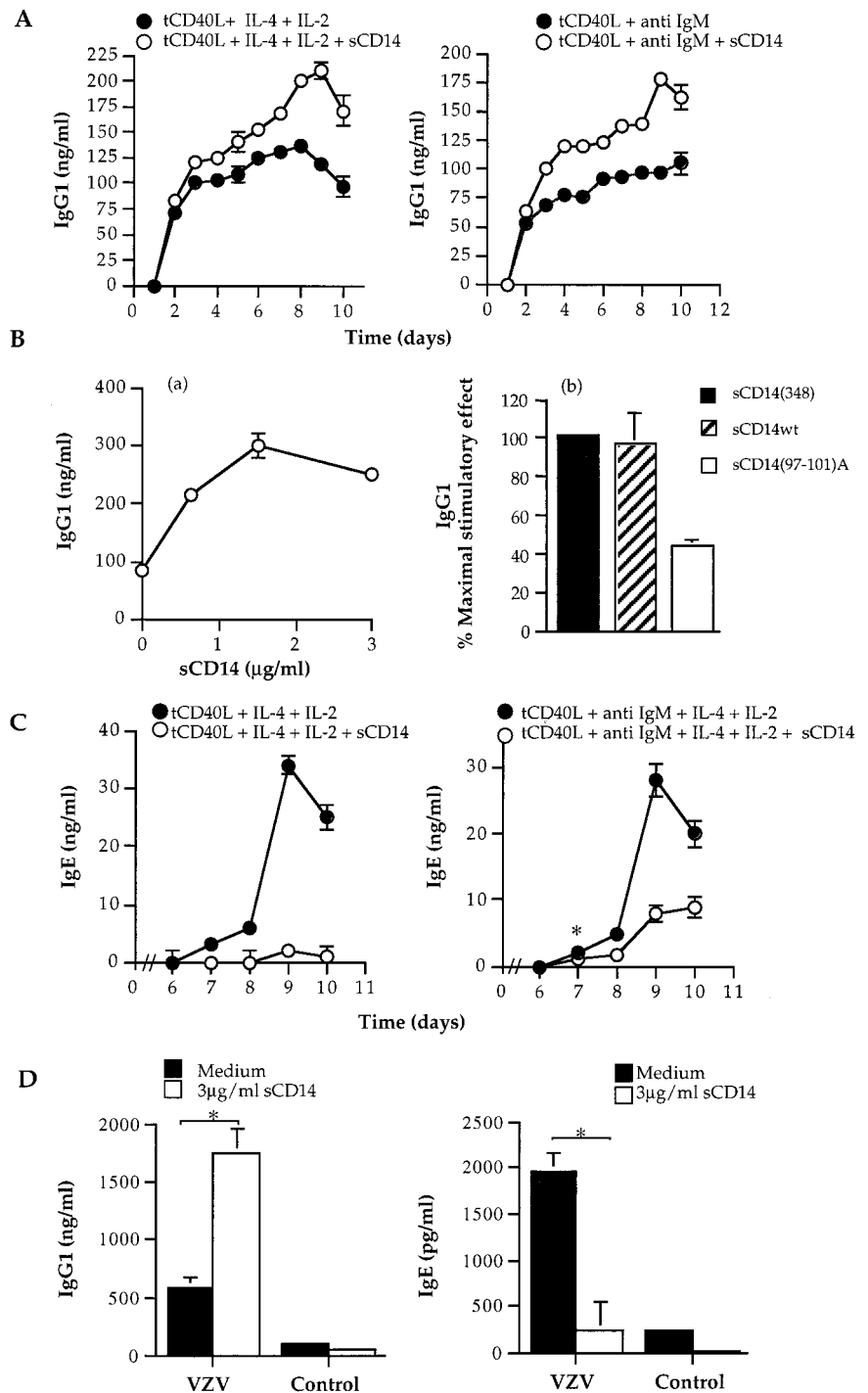


FIGURE 2. Differential effect of sCD14 on IgG1 and IgE production. Time course of IgG1 (A) and IgE (C) released by tonsillar B cells stimulated in the absence or presence of sCD14. Results are means of triplicate cultures of one of four independent experiments with three different tonsil B cell preparations. Differences were significant (Student's *t* test), $p \leq 0.01$; *, Not significant. **B, a**, Dose-dependent stimulatory effect of sCD14 on IgG1 production by tonsillar B cells stimulated with tCD40L + IL-4 + IL-2 for 9 days. Shown is the average of two experiments run in duplicate. **B, b**, Comparison of the stimulatory effect on IgG1 production of sCD14₍₃₄₈₎ with that of sCD14wt and sCD14 (97–101)A mutant. The stimulatory capacity of sCD14₍₃₄₈₎ ($87\% \pm 8.6$, $n = 3$) was arbitrarily set at 100%. Cells were stimulated for 9 days with tCD40L + anti IgM Ab $\pm$ sCD14. **D**, Production of IgG1 and IgE at day 9 by PBMC stimulated with VZV in the absence or presence of sCD14. Results are means of triplicate cultures of one of three experiments. *, $p < 0.01$.

I κ B- α analysis

B cell lines (5×10^5 cells/well) were stimulated by coculturing with tCD40L or tCD32 cells as described above, in the presence or absence of sCD14 (3 μ g/ml). At the indicated time points, cytoplasmic extracts were prepared, equal amounts (40 μ g) run on 15% SDS-PAGE, and analyzed by Western blotting with an anti-I κ B- α Ab (FL Ab, Santa Cruz, CA) as described (12).

Results

Differential binding of sCD14 to B cell subsets

We consistently observed that, depending on the donor, between 24 and 60% of the CD19⁺ cells, but not resting CD3⁺ cells in PBMC, bound sCD14 (Fig. 1A). Tonsillar B cells were stained with the activation marker CD38 and also tested for sCD14 bind-

ing. sCD14 bound 45–92% (depending on the donor) of CD38^{ve/low} B cells and a smaller proportion ($\leq 45\%$) of CD38^{ve/high} cells (Fig. 1B). Tricolor flow cytometric analysis (Fig. 1C) demonstrated that sCD14 bound CD38^{ve/low}, IgD⁺, and IgD[−] B cell subsets as well as CD38^{ve/high}, IgD⁺, and IgD[−] subsets. This phenotype marker expression is consistent with that reported for naive (CD38^{ve/low}, IgD⁺), memory (CD38^{ve/low}, IgD[−]), and germinal center (GC) (CD38^{ve/high}, IgD⁺, and IgD[−]) B cells (15). Naive and IgD⁺ GC cells showed the highest percentage of sCD14 binding, (48.7 and 56.2%, respectively, in Fig. 1C); however, sCD14-binding IgD⁺ GC cells represented a small percentage (11.2%) of the CD38^{ve/high} cells. Binding of sCD14

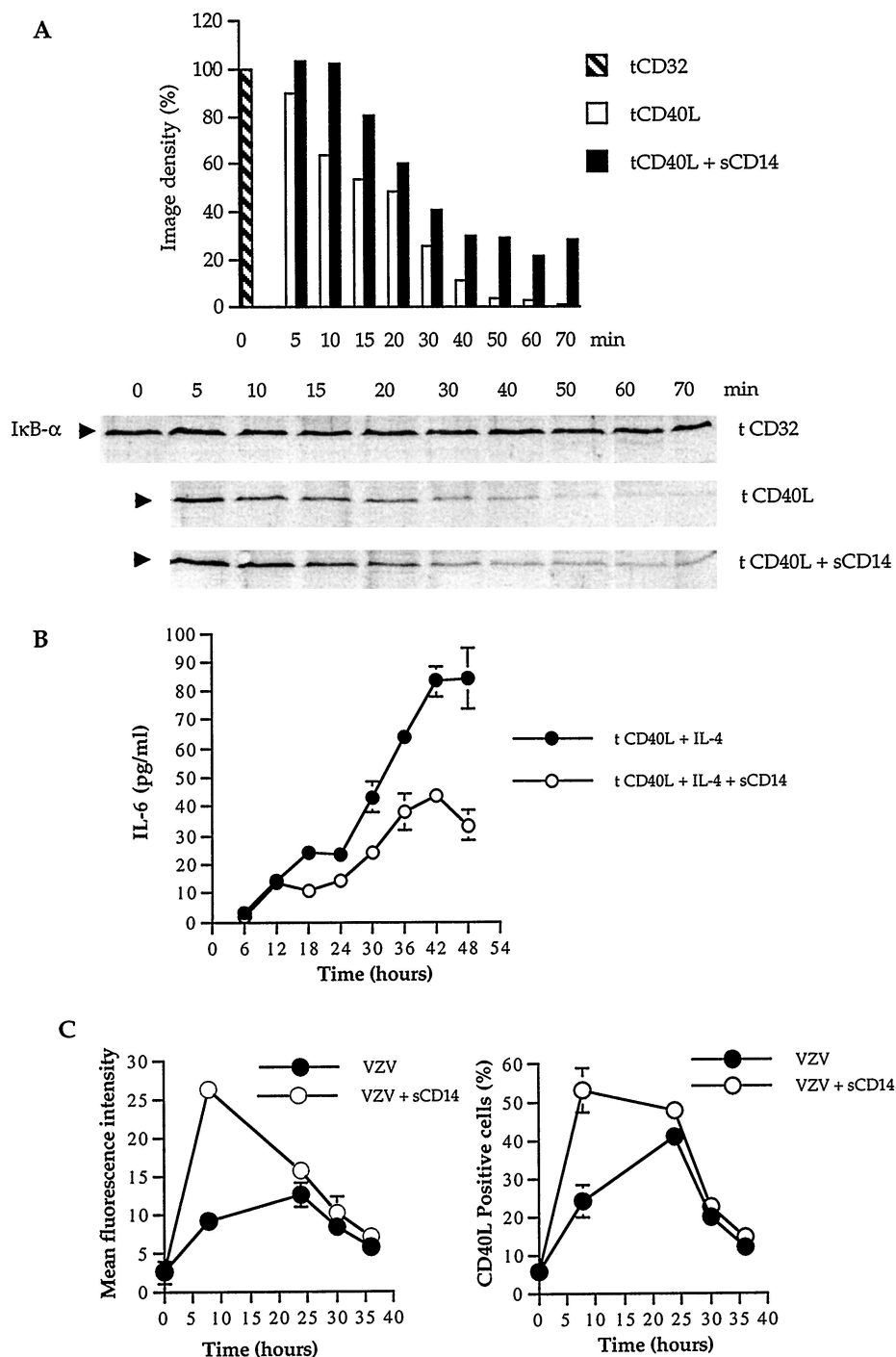


FIGURE 3. sCD14 affects the cognate T-B cell interaction and B-cell derived IL-6 production. **A**, Western blot analysis of the kinetics of I κ B- α degradation in human B cell lines (shown, Akata cell line) stimulated by coculturing with tCD40L cells $\pm$ sCD14. The basal level of I κ B- α was monitored in B cells cocultured with tCD32 cells. Time 0, level of I κ B- α in B cells just before coculturing. The relative density of each image was expressed as percentage of the control (tCD32, set at 100%). Shown is a representative experiment of four. **B**, Time course of IL-6 released by total tonsillar B cells stimulated in the absence or presence of sCD14. Results are means of duplicate cultures of one of eight experiments. **C**, Kinetics and level of CD40L expression in PBMC stimulated with VZV in the absence or presence of sCD14. CD40L expression in T cells was tested by flow cytometry. Results are means of three experiments.

to B cells was saturable and the sCD14 (91–94, 96)A, but not sCD14 (97–101)A, mutant showed reduced binding (Fig. 1D), indicating specific interaction of sCD14 with a putative cellular receptor.

Interaction of sCD14 with activated tonsillar B cells and PBMC results in higher levels of IgG1 and inhibition of IgE production

Total tonsillar B cells were stimulated by culturing on surrogate-activated T cells (tCD40L cells) in the presence of either anti- μ Ab-coated beads or the T cell cytokines IL-4 and IL-2; the cultures were supplemented or not with sCD14 (3 μ g/ml) and tested for IgG1 production (Fig. 2A). At every time point, IgG1 production was significantly increased in the presence of sCD14, irrespective

of the B cell stimulating condition. The magnitude of this effect depended on the donor and was sCD14 dose dependent (Fig. 2B, a). We also tested the effect of sCD14 on IgE production by activated tonsillar B cells (Fig. 2C). Surprisingly, the time course of IgE production showed a strong inhibitory effect of sCD14 irrespective of the stimulatory conditions.

In control experiments, we compared the effect of sCD14₍₃₄₈₎, used in these experiments with that of sCD14wt, and the sCD14 (97–101)A mutant, which we demonstrated does not affect T cell function (12) (Fig. 2B, b). sCD14wt and sCD14₍₃₄₈₎ showed similar capacity to stimulate IgG1 production, whereas sCD14 (97–101)A showed the stimulatory capacity reduced by ~60%. These results indicated that use of the truncated form of sCD14 is

not critical for its effect on B cells and that this is a genuine effect of sCD14. Of note, sCD14 (97–101)A binds to B cells (Fig. 1D), indicating that regions involved in binding and cell signaling are different.

We asked whether T-dependent Ig production by Ag-stimulated PBMC is affected by sCD14. Fig. 2D shows significant increase in IgG1 and profound inhibition of IgE production when PBMC were stimulated in the presence of sCD14. Importantly, the sCD14-treated cultures showed reduced levels of IL-2 (not shown), in agreement with our previous observations (12).

Kinetics of CD40-triggered degradation of I κ B- α is slowed by sCD14

We studied in human B cell lines the kinetics of CD40-triggered degradation of I κ B- α (Fig. 3A), which we reported is affected by sCD14 in T cells (12). CD40 ligation in B cells by tCD40L, but not by tCD32 cells, induced a progressive and marked reduction in the level of cytoplasmic I κ B- α , which started after 5 min, and by 50 min the I κ B- α polypeptide was almost undetectable. CD40-activated B cells in the presence of sCD14 showed a slower kinetics of degradation starting after 15 min, and by 50 min I κ B- α was still detectable. Of note, CD40 B cell-surface expression was not affected by sCD14 (data not shown).

sCD14 inhibits IL-6 production by tonsillar B cells

IL-6 plays an important role in Ig production by increasing the CD40- and IL-4-dependent IgE synthesis and promoting Th2 skewing (16–18). We tested whether sCD14 affects the CD40- and IL-4-induced IL-6 secretion by tonsillar B cells (Fig. 3B). The kinetics of IL-6 release in the presence or absence of sCD14 was similar; however, the level of IL-6 at every time point was lower in the sCD14-treated cultures.

CD40L expression is affected by sCD14

The transient expression of CD40L was followed over the time in Ag-stimulated PBMC in the presence or absence of sCD14 (Fig. 3C). Maximal CD40L expression and percentage of CD40L-expressing cells were observed after 8 h and 24 h in the presence and absence of sCD14, respectively, and were about 2-fold and 30% higher, respectively, in the sCD14-treated cultures; these cultures also showed higher IgG1 and reduced IgE levels, as shown in Fig. 2D.

Discussion

Here we report previously unrecognized activities of sCD14, namely its capacity to bind to different B cell subsets and enhance IgG1 while suppressing IgE production by activated tonsillar B cells and Ag-stimulated PBMC. Furthermore, we obtained insight into the mechanism responsible for the effect on B cells by demonstrating that sCD14 interferes with CD40 signaling in B cells, inhibits IL-6 production by tonsillar B cells, and increases the kinetics and magnitude of CD40L expression on T cells.

sCD14 bound preferentially to CD38^{−ve/low} B cells. This binding profile was consistent with that of B cells in PBMC, because here mainly CD38^{−ve/low} resting B cells (naive and memory cells) are present. However, all main tonsillar B cell subsets bound sCD14, suggesting that non-Ag-selected and Ag-specific B cells are targets for the sCD14 regulatory activity, thus securing control of primary and secondary immune responses.

The interference with the CD40 signaling pathway should, at least, contribute to the regulatory effect of sCD14 on Ig production. Especially in view of the role that signaling through CD40 has in B cell activation, isotype switching, and memory and plasma cell differentiation. Regarding the latter, it has been demonstrated that

interruption/blockage of CD40 signaling favors plasma cell differentiation (19); this would explain the high level of IgG1 in the sCD14-treated cultures. However, the mechanism by which sCD14 increases IgG1 while inhibiting IgE production deserves further investigation. Of note, in experiments not shown here, sCD14 did not affect a number of CD40-mediated B cell responses including proliferation or CD23, CD86, and very late Ag-4 up-regulation (2). These processes would entail induction of an array of transcription factors. It is tempting to speculate that sCD14 interferes preferentially with those CD40-mediated signaling pathways related to Ig class switching. However, the CD40-mediated production of IL-6 was affected. This may contribute to the inhibition of the CD40- and IL-4-induced IgE synthesis.

sCD14 also regulated Ig production by Ag-stimulated PBMC. In addition to the direct effect on B cells, the sCD14-induced reduction of the Th1-like cytokines IL-2 (not shown) and IFN- γ (12), the concomitant reduced T-cell proliferation (12), together with the putatively increased costimulation via CD40L, a consequence of its increased expression in T cells (Fig. 3), may favor humoral immune response. Moreover, the inhibitory effect on IL-4 (12) and that on IL-6 reported here should contribute to reduce IgE isotype switching, as indicated by the work of Vercelli et al. (17).

In conclusion, these findings show that sCD14 is capable of regulating humoral immune response and, together with the previously reported effects on T cells, support the contention that sCD14 may act as a physiological regulator of the immune response in concert with the activity of other regulatory mechanisms. The CD14-deficient mice model will be ideally suited to test this hypothesis. Furthermore, in light of these findings, it deserves to be evaluated the role that the elevated level of sCD14 plays in a number of pathological states, including HIV-1 infection (9) and systemic lupus erythematosus (20), as well as the significance of the reported inverse correlation between sCD14 levels and total serum IgE (21).

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