Human Cancer Biology

C-Reactive Protein Downregulates TRAIL Expression in Human Peripheral Monocytes via an Egr-1–Dependent Pathway

Paola Secchiero1, Erika Rimondi1, Maria Grazia di Iasio1, Chiara Agnoletto1, Elisabetta Melloni1, Ilaria Volpi1, and Giorgio Zauli2

Abstract

Purpose: To investigate the potential link between C-reactive protein (CRP), a known biomarker of acute and chronic inflammation, and TRAIL, a cytokine which plays a key role in the immune-surveillance against tumors.

Experimental Design: Primary normal peripheral blood mononuclear cell (PBMC) and CD14+ monocytes were exposed to recombinant CRP (1–10 μmol/L). TRAIL expression was analyzed by ELISA and/or by quantitative real-time PCR (qRT-PCR). In parallel, the potential role of the transcription factor Egr-1 was investigated by analyzing its modulation in response to CRP and by transfection experiments.

Results: In vitro CRP exposure induced downregulation of TRAIL expression, both at the mRNA and protein level, in unfractionated PBMC and in purified CD14+ monocytes. TRAIL downregulation was not due to a specific toxicity or to contaminating lipopolysaccharide (LPS), as shown by the lack of induction of monocyte apoptosis and by the inability of the inhibitor of LPS polymyxin B to interfere with CRP activity. Of note, CRP downregulated TRAIL expression/release in CD14+ monocytes also in response to IFN-α, the most potent inducer of TRAIL. At the molecular level, the downmodulation of TRAIL by CRP was accompanied by a significant increase of Egr-1. Consistently, Egr-1 overexpression reduced the baseline levels of TRAIL mRNA, whereas knocking down Egr-1 counteracted the ability of CRP to downregulate TRAIL.

Conclusions: Our findings suggest that a chronic elevation of CRP, which occurs during systemic inflammation and often in patients with cancer, might contribute to promote cancer development and/or progression by downregulating TRAIL in immune cells. Clin Cancer Res; 19(8); 1–11. ©2013 AACR.

Introduction

TRAIL is a TNF family member expressed by different cell types, belonging predominantly to the immune system, either as a type II transmembrane homotrimer or, similarly to other membrane-bound ligands of the TNF superfamily, as a soluble protein, which is detectable in the general circulation under physiologic conditions (1). The best-characterized biologic activity of TRAIL, also known as Apo2 ligand, is represented by the induction of apoptosis in a variety of cancer cell types, whereas most normal cells are spared (2, 3). Despite its role as anticancer molecule, only few studies have previously investigated the levels of circulating TRAIL in patients with cancer before and after treatment with chemotherapy (4) or IFN-α (5), which represents the most potent inducer of TRAIL expression and release (6, 7). On the other hand, several studies have shown that serum TRAIL levels are inversely associated with the outcome and mortality risk in patients with impaired kidney function (8, 9) and cardiovascular disease (10–17), and we have recently shown that low levels of circulating TRAIL are associated to overall mortality in old patients (18). However, although circulating TRAIL is assuming relevance as a potential biomarker for different pathologies (8–18), and growing experimental evidences indicate that TRAIL is a potent modulator of the inflammatory responses (19), the molecular mechanisms controlling the levels of circulating TRAIL have not been deeply investigated.

Previous studies (20–24) have evaluated a potential association of TRAIL with C-reactive protein (CRP), an acute-phase protein that represents a known marker of systemic inflammation. Despite some inconsistencies, as TRAIL has been either positively (20, 21) or negatively (22–24) associated to CRP, the potential association between CRP and TRAIL is of particular interest, also
considering that the association between CRP and cardiovascular diseases is well established (25, 26) and an analogous association between CRP and cancer has been proposed (27–30).

On these bases, the aim of the present study was to investigate the link between CRP and TRAIL by analyzing in vitro the effect of recombinant CRP on the expression of TRAIL in unfractionated peripheral blood mononuclear cells (PBMC) as well as in purified CD14+ monocytes.

Materials and Methods

Cell isolation and cultures

Peripheral blood samples were collected in heparin-coated tubes from healthy human blood donors following informed consent, in accordance with the Declaration of Helsinki, and with approval obtained from the Institutional Review Board of the University-Hospital of Ferrara (Ferrara, Italy) and IRCCS Burlo Garofolo of Trieste (Trieste, Italy). PBMC suspensions were isolated by density-gradient centrifugation and CD14+ monocytes were either positively or negatively selected from total PBMC by using the CD14 microbeads and the monocyte isolation kit II, respectively, and the AutoMACS system (Miltenyi Biotech). Primary endothelial cells were isolated as previously described (31) and cells were used between the third and sixth passage in vitro. For further details, see Supplementary Methods.

Cell treatments

Unfractionated PBMC or purified CD14+ cells were resuspended at a cell density of 10^6 cells/mL before treatment with recombinant human CRP (R&D Systems), recombinant human interleukin (IL)-6 (R&D Systems), recombinant human IFN-α (Peprotech, EC), or with the IL-6 receptor-inhibiting monoclonal antibody tocilizumab (RoACTEMRA; Roche Diagnostic GmbH). To exclude the potential effects of lipopolysaccharide (LPS) contaminant in commercial CRP preparations (<1.0 EU per 1 μg of the protein by the LAL method, corresponding to <100 pg endotoxin/1 μg CRP), in selected experiments cells were treated with CRP in combination with polymyxin B (Calcibiochem) or with LPS (Sigma Chemical), as reported in previous studies of our and other groups of investigators (32, 33).

For evaluation of cell toxicity, at various time points after treatments, cell viability of PBMC and purified CD14+ monocytes was monitored by Trypan blue dye exclusion, whereas apoptosis was evaluated by flow cytometry after Annexin-V/propidium iodide (PI) staining (Alexis Biochemicals), according to the manufacturer’s instructions, and as previously described (34, 35). For the apoptosis assays carried out using the BJAB cell line, cells were seeded in 24-well plates at an optimal cell density of 1 × 10^6 cells/mL in coculture with CD14+ cells (2 × 10^6 cells/well). Before coculture, CD14+ cells were either left untreated or preexposed to IFN-α in the absence or presence of human osteoprotegerin (OPG; R&D Systems).

Biochemical measurements

For the measurement of TRAIL levels in cell culture supernatants and cell lysates, analyses were conducted in duplicate by using specific, commercially available ELISA kit (R&D Systems) in agreement with the manufacturer’s instructions and analyzed at 450 nm using an Anthos 2010 ELISA reader (Anthos Labtec Instruments Ges.m.b.H), as previously described (36). For further details, see Supplementary Methods.

RNA analysis

Total RNA was extracted from cells by using the Qiagen RNeasy Plus Mini Kit (Qiagen) according to the supplier’s instructions. For further details, see Supplementary Methods.

Western blot analysis

For Western blot analyses, cells were harvested in lysis buffer containing 50 mmol/L Tris–HCl, 150 mmol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, and protease inhibitors (Protease inhibitor cocktail; Roche Diagnostics), as previously described (37). Equal amounts of protein (50 μg) for each sample were migrated in acrylamide gels, blotted onto nitrocellulose filters, and probed with the following antibodies: anti-TRAIL (R&D Systems), anti-Egr-1 (Santa Cruz Biotechnology), and antitubulin (Sigma-Aldrich). After incubation with peroxidase-conjugated anti-rabbit or anti-mouse immunoglobulin G (IgG; Sigma-Aldrich), specific reactions were revealed with the ECL Western blotting detection reagent (Amersham Corp.; ref. 38).

Transfections

Purified CD14+ cells (4.5 × 10^6) were resuspended into 0.1 mL of human monocyte Nucleofector solution (Amaxa). Two microgram of plasmid DNA or 1 μg of siRNA were mixed with the 0.1 mL of cell suspension, transferred into a 2.0-mm electroporation cuvette, and nucleofected
using an Amaxa Nucleofector II apparatus, following the manufacturer’s guidelines. For further details, see Supplementary Methods.

Statistical analysis
Data were calculated and shown as mean ± SD. The results were evaluated by using ANOVA with subsequent comparisons by Student t test and with the Mann-Whitney rank-sum test. Correlations between data were estimated using Spearman correlation coefficient. Statistical significance was defined as P < 0.05.

Results
Primary CD14+ monocytes exhibit high levels of expression of TRAIL
Taking into account that the cellular source of circulating soluble TRAIL is incompletely understood, in the first group of experiments we have analyzed the levels of TRAIL expression in different cell types, which are thought to contribute to the production and release of circulating TRAIL (1). For this purpose, we have quantitatively determined the mRNA TRAIL levels in different preparations of unfractionated PBMC, purified circulating CD14+ monocytes (Fig. 1A) and endothelial cells. The highest levels of basal TRAIL mRNA levels were documented in purified CD14+ monocytes, followed by unfractionated PBMC, whereas endothelial cells exhibited the lowest TRAIL expression levels (Fig. 1B). To ascertain whether the steady-state mRNA levels were related to the protein expression of TRAIL, we analyzed cell lysates by using an ELISA assay, taking into account that in our experimental conditions TRAIL ELISA was markedly more sensitive than Western blot analysis for the detection of TRAIL protein and allowed a better quantification. As shown in Fig. 1C, by testing at least 50 μg of total cell lysate, detectable levels of TRAIL were observed in unfractionated PBMC and at a significantly (P < 0.05) higher level in purified CD14+ monocytes, but not in endothelial cells, with a strong correlation (R = 0.9; P < 0.01) existing between the mRNA and protein levels of TRAIL (Fig. 1D). On the basis of these data, we have chosen to carry out the majority of the following in vitro experiments by using purified CD14+ monocytes.

Recombinant CRP promotes a dose-dependent downregulation of TRAIL in the absence of cell cytotoxicity
TRAIL has been either positively (20, 21) or negatively (22–24) correlated to CRP, and a series of in vivo studies have shown that increased plasmatic levels of CRP represent a risk factor for cancer development (27–30, 39, 40).
Figure 2. Transcriptional downmodulation of TRAIL expression in primary CD14^+ monocytes by CRP exposure. Primary CD14^+ monocytes and total PBMC were exposed to recombinant CRP. A, CD14^+ monocytes were either exposed for 24 hours to different concentrations of CRP (white bars) or were exposed to CRP (3 μg/mL) for different time points (black bars), before analysis of TRAIL mRNA levels by qRT-PCR. B, correlation between modulation (expressed as percentage of untreated) of TRAIL mRNA and TRAIL protein assessed in the same cell samples. C, cultures of primary CD14^+ monocytes,
Therefore, we found of interest to evaluate in vitro the effect of recombinant CRP on TRAIL expression, by analyzing both mRNA and protein levels in CD14+ cell cultures. Exposure of freshly isolated CD14+ monocytes to recombinant CRP for 24 hours induced a dose-dependent decrease of TRAIL mRNA levels with a significant (P < 0.05) TRAIL decline being observed starting from CRP concentrations of 1 µg/mL (Fig. 2A). Of interest, in kinetics experiments, a significant downmodulation of TRAIL expression in purified CD14+ monocytes started to be observed as early as 1 hour after CRP treatment (3 µg/mL) with a complete shut-off of the mRNA levels between 24 and 48 hours of treatment (Fig. 2A). Also in this group of experiments, the results of TRAIL protein analysis displayed a clear-cut positive correlation with the TRAIL mRNA data, in terms of modulation, both in dose-dependent and kinetics assays (Fig. 2B). In parallel, in all the experiments, we assessed cell viability after 24 to 48 hours of CRP treatment. As shown in Fig. 2C, CRP (used at the highest concentrations of 10 µg/mL) had minor and not significant effects on CD14+ cell viability, ruling out the possibility that TRAIL downmodulation was an indirect consequence of the cytotoxic effect of CRP. Of note, TRAIL downmodulation induced by CRP exposure was not due to CD14+ cell priming, which can occur due to the positive selection procedure for cell purification, as it was observed also in total PBMC (Fig. 2D). In addition, for some bulky coasts we have analyzed in parallel CD14+ cells purified by both positive and negative selection, confirming that the purification procedure (and thus, the cell priming) does not affect TRAIL modulation induced by CRP (Fig. 2E). Finally, to rule out the potential effect of contaminating LPS in the commercial recombinant CRP preparations used (reported by the manufacturer to be <1.0 EU per 1 µg of the CRP protein), cells were treated in the presence of polymyxin B (10 µg/mL), an inhibitor of LPS, or with LPS (used at the concentration of 300 pg/mL, calculated as maximal possible contamination of the CRP). The lack of significant differences between the TRAIL modulation in the absence or presence of polymyxin B and the minimal effect of LPS on TRAIL expression confirmed the specificity of CRP effects (Fig. 2E).

Of note, CRP was able to induce a strong release of IL-6 in the culture supernatant of CD14+ cells in a dose- (Fig. 3A) and time- (Fig. 3B) dependent way, as opposed to the downregulation of TRAIL. Thus, to exclude the possibility that the CRP-mediated downregulation of TRAIL was mediated by the induction of IL-6, in additional experiments CD14+ monocytes were exposed to recombinant IL-6, used at concentrations similar to those released by CD14+ monocytes in response to CRP. As shown in Fig. 3C, recombinant IL-6 did not affect TRAIL expression. Conversely, CRP still induced a significant downregulation of TRAIL also in the presence of the anti-IL-6 receptor antibody tocilizumab (Fig. 3C). Taken together, these data indicate that IL-6 is not involved in mediating the downregulation of TRAIL upon CRP exposure.

**CRP dose-dependently downregulates the IFN-α–mediated induction of TRAIL in monocytes**

Taking into account that TRAIL represents a well-known transcriptional target of IFN (5–7) and that IFN-α represents an important therapeutic tool against various forms of hematologic malignancies (41, 42), we next analyzed whether CRP affected TRAIL expression also in monocyte cultures stimulated with IFN-α. As expected (5–7), IFN-α induced a significant (P < 0.01) increase of TRAIL mRNA (Fig. 4A). In addition, IFN-α stimulation significantly (P < 0.01) increased the release of soluble TRAIL in the culture supernatant of CD14+ monocytes (Fig. 4B). Similarly to the findings obtained in unstimulated monocytes, CRP dose-dependently counteracted the ability of IFN-α to promote TRAIL expression and release (Fig. 4A and B). Again, the inhibitory activity of CRP was significant (P < 0.05) at concentrations ≥1 µg/mL (Fig. 4A and B). To further investigate the potential clinical significance of CRP-mediated downmodulation of TRAIL, we tested the ability of unstimulated and IFN-α-stimulated monocytes to induce apoptosis in TRAIL-sensitive leukemic cells, in the absence and presence of CRP. For this purpose, we have used BJAB, a well-characterized TRAIL-sensitive B lymphoblastoid cell line. Preliminary experiments revealed that viability of BJAB was unaffected by both recombinant CRP (used up to 10 µg/mL) and IFN-α (used at 1,000 U/mL) for 48 hours (data not shown). Therefore, BJAB were cocultured: (i) with untreated monocytes, and (ii) with monocytes preexposed (for 18 hours) to CRP and IFN-α, used either alone or in combination. As shown in Fig. 4C, background apoptosis of BJAB was increased upon coculture with untreated monocytes, and a further increment of BJAB apoptosis was documented when monocytes were prestimulated with IFN-α. The induction of leukemic apoptosis in cocultures with IFN-α–stimulated monocytes was significantly reduced by the addition of recombinant OPG (Fig. 4C), which acts as neutralizing receptor.
for TRAIL, thus showing a key role of endogenous TRAIL in mediating monocyte-driven apoptosis of BJAB leukemic cells. In keeping with its ability to downregulate the release of soluble TRAIL, recombinant CRP significantly decreased BJAB apoptosis levels in cocultures with both untreated monocytes and IFN-α-stimulated monocytes (Fig. 4C).

CRP downregulates TRAIL through an Egr-1–mediated pathway

By computational analysis [as predicted by SABiosciences’ text mining application and the University of
California, Santa Cruz (UCSC; Santa Cruz, CA) genome browser]. TRAIL regulatory region exhibits several consensus DNA-binding sites for potential transcription factors, including for Egr family members, upstream and within the promoter region (Fig. 5A). In this respect, previous studies conducted in endothelial cell models have suggested that the early transcription factor Egr-1 acts as a negative regulator of TRAIL (43, 44). Therefore, to explore the molecular mechanisms underlying the ability of CRP to downregulate TRAIL in CD14+ monocytes, we first examined whether the modulation of TRAIL upon CRP treatment was correlated to the modulation of Egr-1. In kinetics experiments (Fig. 5B), we observed that the progressive transcriptional downmodulation of TRAIL mRNA was concomitant with a significant (P < 0.05) upregulation of Egr-1 mRNA (starting at 1 hour post-CRP treatment), confirmed also by protein analysis (Fig. 5C). Moreover, in keeping with an important role of Egr-1 in modulating the expression of TRAIL, we observed that treatment of CD14+ monocytes with IFN-α exhibited opposite effects with respect to recombinant CRP not only on TRAIL (induction vs. downmodulation, respectively) but also on Egr-1 (downmodulation vs. induction, respectively) levels (Fig. 5D). Interestingly, the simultaneous treatment with CRP dose-dependently counteracted the modulations of both TRAIL and Egr-1 induced by IFN-α (Fig. 5D).

To further elucidate the role of Egr-1 in modulating TRAIL expression in our experimental model, primary CD14+ monocytes were transfected with a plasmid overexpressing Egr-1 or with an empty control vector (Fig. 6A). Overexpression of Egr-1 induced a significant decrease in the basal levels of TRAIL (Fig. 6A). In parallel experiments, we used siRNAs to attenuate Egr-1 expression. After having obtained a significant downmodulation of Egr-1 expression upon siRNA transfection, as documented by quantitative real-time (qRT-PCR; Fig. 6B), we found that in primary CD14+ monocytes transfected with Egr-1–specific siRNAs, downmodulation of TRAIL mRNA was significantly attenuated in CRP-treated cultures with respect to cells transfected with scrambled control siRNA (Fig. 6B). Although the transfection experiments confirmed the key role of Egr-1 in the downmodulation of TRAIL expression in response to CRP stimulation, the lack of effect of Egr-1 knockdown on baseline TRAIL levels suggests that either baseline TRAIL expression is only modestly regulated by Egr-1 or its upregulation requires a more dramatic Egr-1 knockdown, as indicated by the experiments with IFN-α (as shown in Fig. 5D).

Discussion

CRP is an acute-phase plasma protein that increases during systemic inflammation. CRP is produced primarily in the liver and is regulated by proinflammatory cytokines, especially IL-6. In turn, we have shown here that CRP induces the release of IL-6 by PBMC and purified CD14+ monocytes, envisioning a feed-forward loop between CRP and IL-6. CRP levels in blood are normally very low and difficult to detect in healthy individuals, but increase rapidly with inflammation. Increased CRP concentrations have been reported in many diseases, including cardiovascular diseases, diabetes, inflammatory bowel diseases, arthritis, and many cancers. With respect to the CRP association with cancer (45), the robust association described between circulating levels of CRP and cancer risk suggest alternative possibilities: (i) elevated CRP might have a causal role in carcinogenesis, (ii) elevated CRP levels might be the result of an underlying cancer, and (iii) a third factor, such as chronic inflammation, might increase both CRP levels and the risk of cancer (46). In support of the causality hypothesis, it has been proposed that inflammation-associated oxidative damage could initiate carcinogenesis by causing inactivating mutations in tumor-suppressor genes or might facilitate tumor progression by promoting cell motility, vascular permeability, and angiogenesis (47, 48). Indeed, in a large study conducted on the general population, individuals with CRP levels in the highest versus the lowest quintile had a 1.3-fold increased risk of cancer of any type and a 2-fold increased risk of lung cancer. Moreover, among individuals diagnosed with cancer during the study period, those with a high baseline CRP (≥3 mg/L) had an 80% greater risk of early death compared with those with low CRP levels (<1 mg/L). Accordingly, patients with invasive breast cancer and CRP levels >3 mg/L at diagnosis had a 1.7-fold increased risk of death from breast cancer compared with patients with CRP levels <1 mg/L at diagnosis (30). In this context, our current data showing that CRP significantly downregulates TRAIL expression in both freshly isolated PBMC and in purified circulating CD14+ monocytes envision an additional possibility to support the causality hypothesis: CRP might have a direct role in promoting cancer initiation and/or progression by downregulating TRAIL, which is a key molecule mediating the immune surveillance against different kinds of cancer in experimental models (49, 50).

It is also noteworthy that CRP downregulated TRAIL at the transcriptional level likely through the upregulation of Egr-1. Indeed, in CD14+ monocytes, we observed: (i) exposure to CRP-modulated Egr-1 and TRAIL mRNA expression levels with opposite trends; (ii) overexpression of Egr-1 was associated to downmodulation of baseline TRAIL expression; and (iii) knocking down Egr-1 significantly counteracted the CRP-mediated downregulation of TRAIL. On the other hand, the possibility that the CRP induction of IL-6 was involved in mediating the downregulation of TRAIL was excluded as recombinant IL-6 was unable to affect TRAIL expression when added to primary CD14+ monocytes and the addition in culture of the anti-IL-6 receptor antibody tocilizumab did not affect the ability of CRP to downregulate TRAIL. Recently, it has been proposed that human CRP modulates macrophage activity through the increase of protein kinase C (PKC)-δ (51, 52). Although a recent article has linked PKC-δ activation to Egr-1 expression (53), it is beyond the scope of our present article to investigate the potential role of PKC-δ in downmodulating the expression of TRAIL. Therefore, the potential involvement of PKC-δ in promoting Egr-1 expression and TRAIL downregulation in
Figure 5. Induction of Egr-1 in CD14⁺ cells upon exposure to CRP. Primary CD14⁺ monocytes were treated with CRP (3 μg/mL) and analyzed at the indicated time points. A, prediction of Egr-binding sites in the TRAIL gene. B, simultaneous modulation of TRAIL and Egr-1 mRNA by CRP was analyzed by qRT-PCR. Levels of TRAIL and Egr-1 mRNA, normalized to the housekeeping gene POLR2A, were expressed as fold of modulation with respect to the control untreated cultures set to 1 (time = 0). Data are reported as mean ± SD of results obtained in purified CD14⁺ from 4 different individuals. *, P < 0.05 with respect to the untreated cultures. C, Egr-1 protein levels were analyzed by Western blot analysis. Tubulin staining is shown as loading control. Representative examples of Western blot analysis results, out of 3 independent experiments, are shown. After densitometric analysis, Egr-1 protein levels were expressed as arbitrary units (a.u.). D, following treatment with the indicated concentrations of CRP, primary CD14⁺ monocytes were either left unstimulated (left histograms) or stimulated with IFN-α (right histograms). At 24 hours posttreatments, simultaneous modulation of TRAIL and Egr-1 mRNA by CRP was analyzed by qRT-PCR. Levels of TRAIL and Egr-1 mRNA, normalized to the housekeeping gene POLR2A, were expressed as fold of modulation with respect to the control untreated cultures set to 1. Data are reported as mean ± SD of results obtained in purified CD14⁺ from 3 different individuals. *, P < 0.05 with respect to the untreated cultures; §, P < 0.05 with respect to the cultures treated with IFN-α in the absence of CRP.
primary CD14\(^+\) monocytes is an interesting hypothesis, which will require future studies. Likewise, to better understand the basis for CRP action on TRAIL expression in monocytes, additional studies will be necessary to investigate the potential involvement of cell surface receptors, such as Fc\(\gamma\) receptors CD32 and CD64, which have been previously proposed as mediators of CRP activities in monocytes and endothelial cells (51, 54).

In a pathogenetic perspective, it is noteworthy that CRP was able to downregulate TRAIL expression also in IFN-\(\alpha\)-stimulated primary CD14\(^+\) monocytes. These data are particularly remarkable as IFN-\(\alpha\)-treatment has been previously associated: (i) with an increase of the intracellular TRAIL levels in vitro; (ii) with the secretion of soluble TRAIL in the culture supernatant of PBMC and primary CD14\(^+\) monocytes; and (iii) with increased circulating levels of soluble TRAIL in vivo (5–7). Because IFN-\(\alpha\) represents an important therapeutic weapon against different kinds of hematologic malignancies (41, 42), and TRAIL is known to represent a major mediator of the anticancer activity of IFN-\(\alpha\) (5–7), in future clinical studies it will be interesting to investigate changes in the plasmatic concentrations of both CRP and TRAIL before and after IFN-\(\alpha\) therapy. It will be attracting to monitor simultaneously CRP and soluble TRAIL also before and after biologic therapy, to further evaluate the relevance of the inverse correlation between CRP and TRAIL described in our study in clinical settings.

Figure 6. Role of Egr-1 in the transcriptional downregulation of TRAIL by CRP in CD14\(^+\) monocytes. Egr-1 levels were modulated in primary CD14\(^+\) monocytes by transfection experiments. A, CD14\(^+\) monocytes were transfected either with the control empty plasmidic vector or with the pEgr-1 expression vector. Modulation of Egr-1 and TRAIL mRNA was analyzed by qRT-PCR. Levels of TRAIL and Egr-1 mRNA, normalized to the housekeeping gene POLR2A, were expressed as percentage of modulation with respect to the control cultures set to 100. Data are reported as mean \(\pm\) SD of results obtained in purified CD14\(^+\) from 3 different individuals. *, \(P<0.05\) with respect to the cultures transfected with control DNA. B, CD14\(^+\) monocytes were transfected either with the control scrambled (scr.) or with the Egr-1 siRNA. After transfections, downmodulation of baseline Egr-1 was documented by qRT-PCR and is expressed as percentage of modulation with respect to the control not-transfected cultures (Untr., set to 100). Cells transfected as indicated were left untreated or exposed to CRP (3 \(\mu\)g/mL) and analyzed for levels of TRAIL mRNA. Results were expressed as percentage of TRAIL modulation in CRP-treated cultures with respect to the control cultures, which were set to 100 (hatched line). Data are reported as mean \(\pm\) SD of results obtained in purified CD14\(^+\) from 3 different individuals. *, \(P<0.05\) with respect to scrambled transfected cultures.
In conclusion, our data suggest that long-term suppression of TRAIL expression and release by CRP might contribute to increase the risk of tumors associated to chronic diseases, which are characterized by systemic inflammation and CRP elevation (55–57). However, only in vitro experiments carried out by simultaneously analyzing the levels of CRP and TRAIL in patients with premalignant lesions or overt cancer will reveal whether our in vitro data have clinical relevance.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: P. Secchiero, G. Zauli
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.G. di Iasio, C. Agnoletto, E. Melloni, I. Volpi
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P. Secchiero, E. Rimondi, C. Agnoletto
Writing, review, and/or revision of the manuscript: P. Secchiero, E. Rimondi, G. Zauli
Study supervision: P. Secchiero

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