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Arginase-1 and Ym1 Are Markers for Murine, but Not Human, Alternatively Activated Myeloid Cells

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LETTERS TO THE EDITOR

Arginase-1 and Ym1 Are Markers for Murine, but Not Human, Alternatively Activated Myeloid Cells

In an interesting paper, Scotton et al. (1) reported the effects of IL-13 on the transcriptional profile of human monocytes. Comparing their results with studies analyzing the profile induced by IL-4 in murine macrophages (2, 3), the authors notice some differences, giving as examples up-regulation of Ym1 and arginase-1 in murine macrophages, but not in human monocytes. The authors suggest these differences reflect the fact that IL-4 and IL-13 are highly similar, yet different molecules. Besides overlapping effects with IL-4, IL-13 indeed has documented nonredundant functions and effects (4, 5). However, when comparing results obtained in murine macrophages to human monocytes, differences between monocytes and differentiated macrophages, as well as interspecies differences, may be involved. Indeed, human monocytes and monocyte-derived macrophages exhibit differences in their response to IL-4 and IL-13 (6). Moreover, we and others recently documented that, similar to IL-13, IL-4 does not induce arginase-1 in human monocytes and monocyte-derived macrophages (7, 8), in contrast to murine macrophages, where the arginine metabolism is even one of the discriminative criteria between so-called alternatively and classically activated macrophages (9). Furthermore, in human monocytes, under conditions inducing mannose receptor and macrophage galactose-type C-type lectin, markers for IL-4/IL-13-induced, alternatively activated macrophages (7), we have not detected induction by IL-4 and IL-13 of eosinophil chemotactic cytokine, the closest human homolog of mouse Ym1 by sequence identity (Fig. 1). Overall, next to similarities, murine and human alternatively activated myeloid cells exhibit distinct differences. These should be taken into consideration to make optimal use of information obtained from murine models, and to relate it to the human situation.

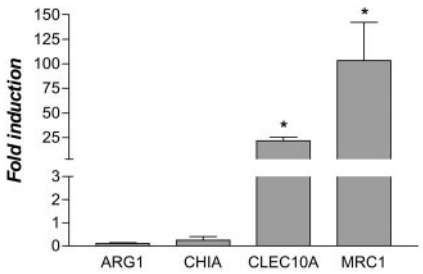

FIGURE 1. In vitro cytokine modulation of ARG1 (Arginase, type I), CHIA (eosinophil chemotatic cytokine), CLEC10A (human macrophage galactose-type C-type lectin), and MRC (mannose receptor, C type 1) expression in human monocytes. Monocytes were isolated from buffy coats of healthy volunteer blood donors and treated with IL-4 plus IL-13 (15 ng/ml each) for 48 h as described (7). Gene expression was determined via quantitative RT-PCR and normalized for the housekeeping gene *GAPDH*. Fold induction of the genes is shown as compared with the no-treatment control (incubated in vitro in the absence of cytokines). Data are shown for one representative experiment. *, Significantly higher than no treatment ($p < 0.05$). The error bars indicate the SEM.

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The Authors Respond

In a recent paper, we reported the transcriptional profile induced by IL-13 in human monocytes (1). We described that in monocytes, IL-13 regulates 142 genes, 85 of which were increased and 57 decreased. The majority of these genes were related to the inflammatory response, innate immunity, and lipid metabolism. Regulated genes included characteristic markers of alternatively activated macrophages, such as the mannose receptor (MRC1), CD23 (FCER2, FcR for IgE), CCL22 (also known as MDC), arachidonate 15-lipoxygenase (ALOX15), IL-1RII, IL-1Ra, and a number of novel genes (1).

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Genes such as *Ym1* and *Arginase*, which are known to be regulated by IL-4 in murine macrophages (2, 3), were not detected in our study. We speculated that these differences may reflect the fact that IL-4 and IL-13 are highly similar yet different molecules (4, 5).

In their letter, Raes and colleagues comment on additional factors that may explain differences in the *IL-4* and *IL-13* gene profiles, including differences related to the type of cells investigated (e.g., blood monocytes vs differentiated macrophages) or related to the species investigated (i.e., human vs mouse). We completely agree with Raes and colleagues, and we also believe that multiple factors may be responsible for the discrepancies reported (1) and different explanations might be found for any single gene investigated. In relation to the differential induction of Arginase in our experimental conditions and those reported by others (2, 3), it is likely that the explanation is in the interspecies differences between human cells and mouse macrophages. In fact, in a gene profile of human monocytes, monocyte-derived dendritic cells and monocyte-derived macrophages polarized to the M1 phenotype (LPS or immunocomplexes) or the M2 phenotype (IL-4 or IL-13) (6), we also have not found an up-regulation of Arginase (our unpublished observations). An integrated analysis of the transcriptomes in different experimental conditions will provide further evidence on the level of regulation of monocyte and macrophage-regulated genes. Therefore, we certainly agree with the note of caution that Raes and colleagues have pointed out.

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Effect of CD3/CD28 Bead-Activated and Expanded T Cells on Leukemic B Cells in Chronic Lymphocytic Leukemia

We read with interest the paper by Bonyhadi et al. (1) published February 15, 2005. We have, however, two concerns.

CD3/CD28 stimulation resulted in successful expansion of T cells from B-CLL PBMC samples. The authors state that this was associated with a rapid decrease in numbers of leukemic B cells. Given the average 1400-fold expansion of T cells seen, a drop in the percentage of B cells is not surprising, but this decrease may only be relative and not necessarily absolute as no actual cell numbers are given.

In addition, we have some misgivings about the apoptosis assay using a PE-conjugated anti-CD19 Ab in association with PI. After excitation with a 488-nm laser, PI fluoresces in the emission spectrums of both the FL2 and FL3 channel. In the system described, gating on the PE^+ cells would therefore include all $CD19^+$ cells and also all PI^+ cells of any lineage, for example T cells. This would result in the apoptosis levels for "$CD19^+$" cells appearing far greater than they actually are. An alternative approach, which we have used, is to use 7-AAD (which fluoresces in the FL3 channel only) as opposed to PI, allowing distinction between $CD19^+$ cells and all other markers. Positively selected T cells from nine separate B-CLL PBMCs were activated overnight with CD3/28 beads (Dynal). These were subsequently cocultured with autologous B-CLL cells at ratios ranging from 1:1 to 1:5 T to B cells. Assessment of apoptosis of the $CD19^+$ and $CD19^-$ populations was performed between 48 and 96 h of culture using FITC-annexin V and 7-AAD. In contrast to the results reported, we found that, in six of nine cases, viability was increased and apoptosis decreased in the $CD19^+$ cells in cocultures compared with PBMCs alone (Table I). We do see a shift toward annexin positivity, but this is in the $CD19^-$ cells (representing T cells) from cocultures as opposed to the $CD19^+$ cells (see Fig. 1).

Table I. *Increased viability and decreased apoptosis in $CD19^+$ cells in cocultures compared with PBMCs alone*[a]

	% Viable Cells		% Apoptosis	
	PBMCs alone	Cocultures	PBMCs alone	Cocultures
48 h				
Case 1	25.3	35.9	74.2	63.3
Case 2	57.8	56.3	41.2	39.4
72 h				
Case 3	76.9	70.1	20.4	27.5
Case 4	13.5	33.0	85.7	65.3
Case 5	38.2	55.4	60.0	41.0
Case 6	69.0	46.5	29.8	51.8
Case 7	55.1	69.0	38.9	28.2
96 h				
Case 8	59.1	79.1	37.6	12.1
Case 9	89.2	92.8	9.4	6.3

[a] Most cases show increased viability and decreased apoptosis of the CD19 fraction in cocultures compared to PBMCs alone. Only cases 3 and 6 show less viable cells and increased apoptosis in cocultures compared to PBMCs.

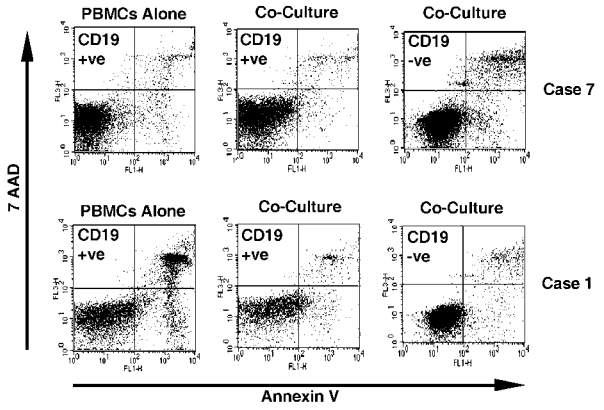

FIGURE 1. Examples of two cases (*upper panel*, case 7; *lower panel*, case 1) using FITC-annexin V and 7-AAD to assess cell viability and apoptosis. In the cocultures, the $CD19^-$ fraction shows a shift to annexin positivity as opposed to the $CD19^+$ fraction.

In conclusion, we do not believe that CD3/CD28-activated T cells cultured with leukemic B cells in B-CLL necessarily results in increased apoptosis of malignant cells, and the increase seen in the work reported by Bonyhadi et al. may be the result of technical factors. Our data would suggest that the opposite may in fact be true.

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The Authors Respond

We appreciate the comments by Drs. Patten, Devereux, and Buggins. It is likely that the differences in their results compared with ours are related to the experimental conditions utilized in their studies.

Dr. Patten et al. state that T cells were positively selected and activated overnight with the CD3/CD28 beads. We have examined the kinetics of activation of CLL patients' T cells with CD3/CD28 beads in detail (1). These studies have shown that induction of the key effector molecule CD154 (as well as others) is delayed compared with T cells from healthy donors. Several days of exposure of T cells from CLL patients to CD3/CD28 beads are required to activate and up-regulate key effector molecules. In our studies, we incubated the patients' T cells with CD3/CD28 beads for 13 days. In contrast, Dr. Patten et al. reported a brief overnight incubation of the patients' T cells with CD3/CD28 beads, which may be insufficient to achieve comparable levels of activation and effector molecule up-regulation. Second, if the beads were not removed following the short overnight incubation as described by Patten et al., they would remain firmly attached to the T cells and provide a physical barrier that could inhibit interaction of T cells with CLL cells, which we have shown in the paper is required for induction of apoptosis of the leukemic B cells. In our studies, a much longer incubation period (13 days) was utilized, which results in several hundred-fold increase in the total number of T cells in the cultures concomitant to the release of the beads from the T cells. In contrast to the experimental conditions of Patten et al., T cells are able to directly interact with CLL cells, thereby inducing CD95 up-regulation and facilitating induction of apoptosis.

With regard to the issue of T cells possibly being counted as B cells undergoing apoptosis due to use of propidium iodide, we too were concerned about this possibility. To rule out this possibility, we labeled the patients' CLL cells with CFSE and subsequently gated on this population to ensure that no T cells (non-CFSE-labeled) would be included in our analyses. The numbers we obtained by this approach were similar to those reported in our paper, so we are confident that our conclusions are correct.

Finally, while not presented in our paper, we have also tracked expression of CD86 and CD95 on leukemic B cells in primary cultures in which CD3/CD28 beads were incubated with PBMC from CLL patients. Leukemic B cells disappeared rapidly between days 6 and 9 of culture. This occurred shortly after CD154 was induced on T cells and CD86 and CD95 induced on tumor cells. We presume leukemic B cells may be killed in a similar manner in vivo and plan to study this possibility in the future.

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