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Eosinophils, but not neutrophils, exhibit an efficient DNA repair machinery and high nucleolar activity

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Funding: this work was funded by MURST-COFIN 2005 and by Associazione Italiana per la Ricerca sul Cancro (AIRC) 2006.

Manuscript received March 2, 2007. Manuscript accepted July 17, 2007.

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ABSTRACT

Background and Objectives

Traditionally eosinophils have been considered terminally differentiated cells that play a role in host protection against parasites. However, there is some evidence showing that eosinophils are, in fact, multifunctional leukocytes involved in inflammatory responses, as well as in tissue homeostasis. We characterized the transcriptome profile of human eosinophils, and, for the purpose of comparison, the transcriptome profile of neutrophils, monocytes and hematopoietic progenitor cells. Moreover, we studied the activation of selected cellular processes for which a significant differential expression was demonstrated.

Design and Methods

We profiled gene expression using Affymetrix GeneChips. DNA repair capacity was tested using the comet assay. Nucleoli and their activity were characterized by transmission electron microscopy analysis, silver staining of nucleolus regions (AgNOR) and RNA staining.

Results

Gene expression profiling showed that eosinophils appear hierarchically closer to monocytes than to neutrophils. Gene ontology mapping of differentially expressed genes revealed that eosinophils express categories very similar to those expressed by monocytes, related to DNA repair and nucleolar functions. Moreover, our data show that eosinophils and monocytes maintain the ability to repair both double and single strand DNA breaks, whereas neutrophils lack this capacity. Furthermore, eosinophils exibit nucleolar activity, which is lacking in neutrophils, but resembles that in monocytes.

Interpretation and Conclusions

The presence of large, active nucleoli in eosinophils, coupled to marked activity of DNA repair systems, suggests that eosinophils are not terminally differentiated cells. Indeed, their transcriptome profile and functional properties are more similar to those of non-terminally differentiated cells such as monocytes, rather than to neutrophils.

Key words: eosinophils, gene expression profile, DNA repair, nucleolar function, myeloid differentiation.

Haematologica 2007; 92:1311-1318. DOI: 10.3324/haematol.11472

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Eosinophils are now known to be multifunctional leukocytes involved in the pathogenesis of numerous inflammatory processes, including parasitic helminth infections and allergic diseases.[1] In response to diverse stimuli, eosinophils are recruited from the circulation into inflammatory foci, where they modulate immune responses through an array of mechanisms. Eosinophils can release numerous cytokines, chemokines and lipid mediators.[2,3] These molecules have pro-inflammatory effects, including upregulation of adhesion systems, modulation of cellular trafficking, regulation of vascular permeability, mucus secretion and smooth muscle contraction. Eosinophils can trigger antigen-specific immune responses by acting as antigen-presenting cells. Furthermore eosinophils can serve as major effector cells inducing tissue damage and dysfunction by releasing toxic granule proteins and lipid mediators.[2,3] In recent years, eosinophils have been shown to be involved in numerous other biological processes, including post-pubertal mammary gland development,[4] estrus cycling,[4,5] reactions to organ transplantation,[6] allergic inflammatory responses[7] and neoplasia.[8]

Some investigators have shown that eosinophils are implicated in airway epithelium remodeling.[9] Eosinophils are capable of producing a wide range of cytokines involved in tissue remodeling such as transforming growth factor (TGF)-α, TGF-β1 and vascular endothelial growth factor (VEGF), and evidence from animal models supports the key role of eosinophils in this process: in fact, deletion of the interleukin-5 gene[10] has been shown to suppress lung eosinophilia and airway remodeling in murine models of allergic asthma.[11] In inflammatory conditions, including asthma and inflammatory bowel disease, localization of eosinophils to nerves is associated with plasticity, specifically nerve cell remodeling.[12] Eosinophil adhesion to nerve cells, via nerve cell intercellular adhesion molecule-1, results in an adhesion-dependent release of granule proteins including the major basic proteins (MBP) and eosinophil-derived neurotoxin. These granule proteins released from eosinophils may affect nerve cell signaling and survival, leading to nerve cell remodeling. In particular, MBP1 may regulate peripheral nerve plasticity through the inhibition of apoptosis.

Although, as a whole, eosinophils have historically been considered end-stage cells involved in host protection against parasites, numerous lines of evidence have now changed this perspective by showing that eosinophils are pleiotropic multifunctional leukocytes involved in the initiation and propagation of diverse inflammatory responses, as well as modulators of adaptive immunity and tissue remodeling. Over the past 10 years, global gene expression profiling has changed the way of investigating the biology of tissue cells. The capacity of microarray information has increased consistently, so that expression of the entire transcriptome can now be measured. These data, along with functional annotations of the genome, provide researchers with global information about the transcriptional activation or down-regulation of specific functional modules. Very few studies have been published so far about the gene expression profile of eosinophils, and all studies that have been published are focused on the molecular mechanisms underlying the eosinophils' involvement in atopic or inflammatory diseases.[3] No data are available about the molecular phenotype of circulating eosinophils, nor about the specific differences between the gene expression profile of eosinophils and the transcriptome profiles of mature leukocytes. To gain new insights into the molecular and functional properties of eosinophils, we investigated the molecular phenotype of human eosinophils and compared it with that of neutrophils, monocytes and $CD34^+$ hematopoietic progenitor cells.

Design and Methods

Cell populations

To obtain peripheral blood $CD34^+$ hematopoietic progenitor cells, 12 healthy donors were given glycosylated recombinant human granulocyte colony-stimulating factor (rh G-CSF) (lenograstim, Rhone-Poulenc Rorer, Milan, Italy) administered subcutaneously at a dose of 10 μg/kg/day for 5-6 days. Progenitor cell purification and phenotypic analysis were performed as previously described.[12] Monocytes ($CD14^+$ cells), neutrophils ($CD16^+$) and eosinophils ($CD16^-$) were isolated from the peripheral blood of 12 healthy donors using magnetic cell-sorting procedures. Briefly, the peripheral blood was subjected to centrifugation on a Ficoll-Hypaque gradient; $CD14^+$ cells were purified from peripheral blood mononuclear cells utilizing a CD14 MicroBeads kit (Miltenyi Biotech, Auburn, CA, USA). Simultaneously, $CD16^+$ and $CD16^-$ cells were isolated by positive selection or depletion, respectively, from the pellet using CD16 MicroBeads (Miltenyi Biotech, Auburn, CA, USA).

Aliquots of purified Lin^+CD34^+, $CD14^+$, $CD16^+$ and $CD16^-$ cells were reanalyzed on a Coulter Epics XL flow cytometer to assess their purities which were 98.2±0.4%, 97.2±0.5%, 98.5±0.7% and 99.0±0.6%, respectively. For differentiated myeloid cells, flow cytometric data were confirmed by morphological analysis (*Online Supplementary Figure 1*).

RNA extraction and microarray data analysis

Total RNA was isolated from each cell population (2×10^5 cells) from each donor using a modification of the guanidinium isothiocyanate procedure and ultracentrifugation on a cesium chloride gradient.[12] RNA samples originating from 12 donors were pooled in order to obtain at least 2 μg per sample.

The biotin-labeled target synthesis reactions as well as the Affymetrix HG-U95Av2 GeneChip (Affymetrix, Santa Clara, CA, USA) arrays' hybridization, staining and scanning were performed as previously described.[12]

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Differentially expressed genes were selected, as the sequences showing a change call *I* or *D*, using the GeneChip Operating Software (GCOS) comparison analysis algorithm in 100% of the pair-wise comparisons between each differentiated myeloid cell population and the other samples and a signal log ratio of at least one (2-fold change) (*Supplementary Table 3*). The generated list and, independently, the GCOS generated absolute analysis data were uploaded onto GeneSpring™ software version 7.2 (Silicon Genetics, Redwood City, CA, USA) in order to perform clustering analysis. To identify the gene ontology (GO) categories characterized by significant numbers of genes differentially expressed in each cell population we utilized an accessory program of GenMAPP 2.0 software, MAPPFinder 2.0β.[13]

DNA repair functional assay (comet assay)

Alkaline and neutral single-cell gel electrophoresis (comet assay) was performed to detect single- and double-strand DNA breaks, respectively. The comet assay was carried out using the Trevigen comet assay kit (Trevigen Inc., Gaithersburg, Maryland, USA), according to the manufacturer's instructions. CD34$^+$ cells, monocytes and granulocytes were treated for 2 hours at 37°C with 200 μM methyl methanesulfonate (MMS) to induce single-strand DNA breaks or with 0.2 μM mitoxantrone to induce double-strand breaks, then washed twice with ice-cold phosphate-buffered saline, and finally rinsed with RPMI/10% fetal bovine serum. Alkaline and neutral comet assays were performed immediately after these treatments and 6 hours after the removal of the MMS or mitoxantrone. Samples were stained with the supplied SYBR green dye, and the slides were viewed using a Zeiss Axioskop 40 epifluorescence microscope. To ensure random sampling, 50 images/slide were captured and the comet moment in each cell was quantified using Scion Image software (Scion Corporation) to outline each comet head and tail manually, then integrating the SYBR fluorescence within the outline. The comet moment was calculated using the following equation described by Kent *et al.*:[14] comet moment= $\Sigma_{0\text{-}n}$ [(intensity of DNA at distance X) × (distance)]/intensity of total DNA. The mean comet-moment value obtained from control samples was subtracted from the mean comet-moment value for each drug treatment. Data shown are the mean values from five independent experiments performed on samples from five different donors. The percentage of remaining MMS- or mitoxantrone-induced damage was calculated by comparison with the total score (100%) of initial DNA damage induced by MMS or mitoxantrone treatment [15]

Transmission electron microscopy (TEM) analysis

Cells were centrifuged at 12000 × g for 5 min at 10°C and routinely processed for ultrastructural analyses.[16] Morphometric analysis of CD16$^+$, CD16$^-$, CD14$^+$ and CD34$^+$ cells was performed on at least 50 micrographs for each cell type.[16]

Staining for nucleolar RNA and nucleolus organizer regions (NOR)

Nucleoli were investigated in cytospins of CD34$^+$ stem/progenitor cells, monocytes, neutrophils and eosinophils purified from the peripheral blood of five healthy donors. Nucleoli were visualized by cytochemical procedures for the demonstration of RNA and silver stained proteins of NOR. In order to detect RNA-containing structures, cytospins were stained with buffered methylene blue without prior fixation.[17,18]

Silver staining was performed to detect active NOR according to a previously described procedure.[19]

Results

The mRNA expression of phenotype-specific markers reveals the high purity of the sorted populations

We assessed gene expression in all cell populations using the Affymetrix HG-U95Av2 GeneChip array, representative of 12625 transcripts. All the data have been deposited in the MIAME compliant Gene Expression Omnibus (GEO) public database, at *http://www.ncbi.nlm.nih.gov/geo*. The GEO accession numbers are reported in *Supplemental Table 2*.

In order to evaluate the purity of sorted cell populations more stringently, we initially looked for expression of a large number of genes, e.g. surface markers, immune defense proteins and transcription factors that are associated with neutrophils, monocytes and eosinophils. *Supplemental Table 2* shows the results of this analysis revealing that each cell population preferentially expresses the transcripts related to the respective phenotype; in fact, the data are consistent with results already published in literature (*Supplemental Table 2*).

Eosinophils appear hierarchically closer to monocytes than to neutrophils

Figure 1 shows the results of unsupervised clustering analysis obtained using the condition tree option included in the GeneSpring package and applying Pearson's equation as a correlation measure. Interestingly, the clustering shows that, despite their commonly described morphological and physiological characteristics, eosinophils appear hierarchically closer to monocytes than to neutrophils. Next we addressed the transcriptome differences between the three differentiated phenotypes, i.e. monocytes (CD14$^+$), neutrophils (CD16$^+$), and eosinophils (CD16$^-$). Using the filtering procedures described in the Design and Methods section, we selected a list of 2290 probesets (*Supplemental Table 3*) showing at least a two-fold difference in one differentiated phenotype vs all the other cell populations. Moreover, the gene list of 2209 probesets was uploaded onto MAPP

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Finder 2.0 software to identify prevalent categories in the GO list of families of biological processes. The GO analysis revealed that the genes preferentially expressed by eosinophils were mapped in functional categories very similar to those of genes preferentially expressed by monocytes. In fact, *Supplemental Table 4A* and *C* shows that prevalent categories increased in CD16$^-$ and CD14$^+$ cells were involved in nucleolus metabolism (e.g. *nucleolus organization and biogenesis*, *nucleologenesis*, *nucleolus to nucleoplasm transport*), as well as in DNA repair mechanisms (e.g. *DNA repair*, *positive regulation of DNA repair*, *DNA ligation during DNA repair*). Conversely, the prevalent categories up-regulated in CD16$^+$ were *defense response*, *immune response*, *response to biotic stimulus*, *cell cycle arrest*, and *negative regulation of DNA repair* (*Supplemental Table 4B*). In the light of these results, we next studied the processes of DNA repair and nucleolar metabolism in detail.

Eosinophils maintain the ability to repair both double- and single-strand DNA breaks

Genes involved in all DNA repair systems (base excision repair, nucleotide excision repair, mismatch repair, double-strand break repair) exhibited, in agreement with their kinetics and differentiation status,[20] a preferential expression in CD34$^+$ cells with some interesting exceptions. It is, in fact, worth noting that CD16$^-$ and CD14$^+$ cells conserve the expression of several DNA repair genes, belonging to all cellular DNA repair systems (Figure 2), while CD16$^+$ cells do not. Next, in order to confirm that the DNA repair capacity of differentiated myeloid phenotypes differs from that of CD34$^+$ cells, we tested the cells' capacity to repair MMS-induced single-strand DNA breaks[21] and mitoxantrone-induced double-strand DNA breaks,[22] using alkaline and neutral single-cell gel electrophoresis, respectively (comet assay). As shown in Figure 3, the tail length, intensity and shape differed according to the cell type; in particular, compared to CD34$^+$ cells, monocytes and eosinophils, neutrophils showed a higher degree of DNA damage induced by either mitoxantrone or MMS. Six hours after removal of the drug CD34$^+$ cells showed a reduction of approximately 80% in both mitoxantrone- and MMS-induced DNA damage, whereas CD16$^-$ and CD14$^+$ cells showed a reduction of about 60% in both double- and single-strand DNA breaks. Conversely, the comet moment of neutrophils was only slightly decreased compared with the same cells at T0 (20% reduction in DNA damage both the double and single strand comet assays) (Figure 3, panels a-d).

These data confirm that eosinophils and monocytes maintain the ability to repair both double and single strand DNA breaks, whereas neutrophils lack this capacity.

Eosinophils show a nucleolar activity closer to CD34$^+$ cells than to CD16$^+$ cells

The global expression analysis of genes involved in RNA transcription (Figure 2A and *Supplemental Figure 2B*), splicing, capping and polyadenylation (*Supplemental Figure 2C*) showed that the process of RNA maturation is mainly active in CD34$^+$ cells; as far as concerns myeloid differentiated cells, CD14$^+$ and CD16$^-$ cells showed a higher expression of these genes, as compared with CD16$^+$ cells.

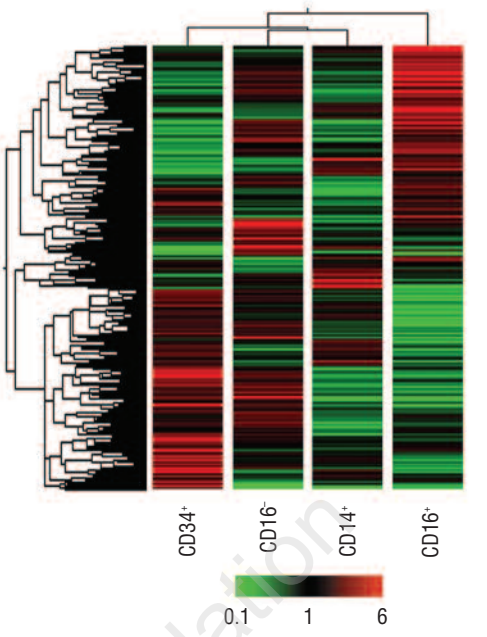

Figure 1. Unsupervised clustering analysis. Clustering was performed using an unsupervised approach and applying Pearson's correlation equation. A combination of two hierarchical clustering analyses (gene tree and condition tree) is shown. Gene coloring was based on normalized signals as shown at the bottom of the figure.

Moreover, analysis of the expression of genes coding for ribosomal proteins demonstrated that these transcripts, which are highly expressed in CD34$^+$, CD16$^-$ and CD14$^+$ cells, undergo a remarkable down-regulation in CD16$^+$ cells (*Supplemental Figure 3A*). Consistently, the same expression pattern was found for genes coding for nucleolar proteins (Figure 4) and genes involved in protein translation and modification (*Supplemental Figure 3B*).

It has been demonstrated that nucleolar size and the distribution of main nucleolar components, such as nucleolar RNA-containing structures and silver-stained nucleolar organizer regions (AgNOR), correlate with nucleolus activity.[23] Moreover, the differentiation and maturation of blood cells is accompanied by a decrease in nucleolar size and the conversion of large nucleoli to ring-shaped nucleoli and finally to micronucleoli, which reflect the decrease of nucleolar activities.[24] In order to demostate the presence of different patterns in nucleoli structure and activity, we performed TEM and AgNOR analyses.

At the ultrastructural level (Figure 5), nucleoli were clearly visible in all cells types (Figure 5A,B,C,D) and frequently observed in close contact with the chromatin adjacent to the nuclear membrane. At higher magnification (Figure 5 a, b, c, d,), both filamentous and the granule nucleolar components were recognized in CD34$^+$ (a), CD14$^+$ (b) and CD16$^-$ (c) cells, but not in CD16$^+$ cells (d). Nucleolar segre-

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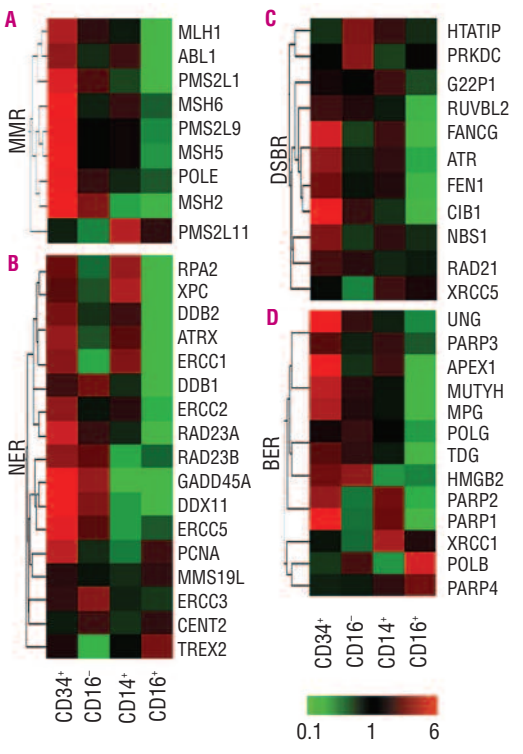

Figure 2. Expression of genes involved in DNA repair. Eisen tree map computed using the GeneSpring *gene* tree and Pearson's correlation equation on the modulated probe sets belonging to the following categories: mismatch repair (MMR, Panel A), nucleotide excision repair (NER, Panel B), double-strand breaks repair (DSBR, Panel C), base excision repair (BER, Panel D). The signal-based coloring legend is shown at the bottom of the figure.

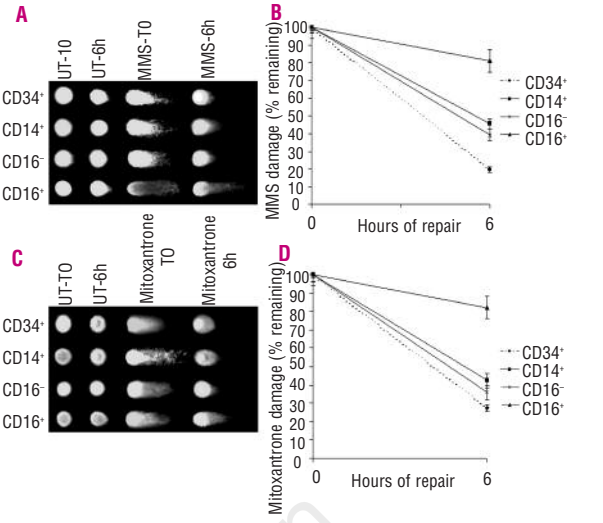

Figure 3. Comet assay. Representative images were taken from alkaline (Panel A) or neutral (Panel C) comet assays of CD34$^+$, CD14$^+$, CD16$^-$ and CD16$^+$ cells before treatment (UT), immediately after MMS or mitoxantrone treatment for 2 hours (T0), and 6 hours after removal of MMS or mitoxantrone (6h). Panels B and D: Median relative tail moment of more than 50 cells for each data point, calculated by comparison with the total score (100%) of initial DNA damage induced by MMS (Panel B) or mitoxantrone (Panel D) treatment. The results reported are representative of five independent experiments. Mean values±2 SEM (confidence range 95%) were: MMS (Panel B): CD34$^+$: 19.8±1.71; CD14$^+$: 45.4±2.95; CD16$^+$: 81±6.37 CD16$^-$:39.2±3.3. Mitoxantrone (Panel D): CD34$^+$: 19.8±1.85; CD14$^+$: 45.4±3.42; CD16$^+$: 81±5.37; CD16$^-$:39.2±3.73.

gation, which is the separation and migration of the filamentous from the granular component, was frequently observed in CD16$^+$ cells (Figure 5d). It has been demonstrated that nucleolar segregation produces a decrease in the activity of RNA polymerase[25] and it is, therefore, conceivable that these changes might be evident in the nucleolus when cells have ceased to be active.

Morphometric analysis (Figure 6) showed that the surface area covered by nucleoli progressively decreased in CD14$^+$, CD16$^-$ and CD16$^+$ cells compared to in CD34$^+$ cells (Figure 6a); by contrast, the ratio of marginated versus centrally located nucleoli showed the opposite trend, with CD16$^+$ cells being the cell type with the highest number of centrally located nucleoli (Figure 6b). Our analysis shows that CD16$^-$ cells had numerous nucleoli in close contact with the chromatin in proximity of the nuclear membrane, (Figure 5C,c; Figure 6b), similarly to CD14$^+$ cells (Figure 5B, b; Figure 6b). In contrast, nucleolar margination against the inner membrane of the nuclear envelope was less evident in CD16$^+$ cells (Figure 5D, d; Figure 6b).

The phenomenon of nuclear margination has led many observers to speculate that such arrangement facilitates nucleous-cytoplasmic exchanges within the nucleus in states of active protein synthesis.[26]

AgNOR analysis showed that, after staining for nucleolar silver-stainable proteins (SSP), nucleoli were detected only in CD34$^+$ cells, monocytes and eosinophils, whereas they were undetectable in neutrophils. The nucleoli of CD34$^+$ cells appeared as clusters of silver-stained particles, while in monocytes and in eosinophils they appeared mostly as small single black SSP (*Supplemental Figure 4*). In CD34$^+$ cells the number of nucleoli ranged between two to five, with the mean value of the nucleolar coefficient being 3.25±0.4 (*Supplemental Figure 4A*). The nucleolar coefficients decreased to 2.1±0.3 and 1.6±0.2 in monocytes and eosinophils, respectively, (*Supplemental Figures 4B and 4C*) whereas neutrophils did not contain AgNOR-detectable nucleoli (*Supplemental Figure 4D*). Monocytes and eosinophils contained prevalently micronucleoli and, less frequently, ring-shaped nucleoli, while large nucleoli were very rare.

After staining for RNA-containing structures, CD34$^+$ cells showed large nucleoli with a homogeneous distribution of RNA, while CD16$^-$ and CD14$^+$ cells showed mostly uniform staining of micronucleoli. In agreement with the AgNOR analysis, neutrophils were negative for RNA staining (*data not shown*).

Discussion

The two professional phagocytic cell types, neutrophils and macrophages, develop from a common progenitor cell called a granulocyte/macrophage progenitor cell.

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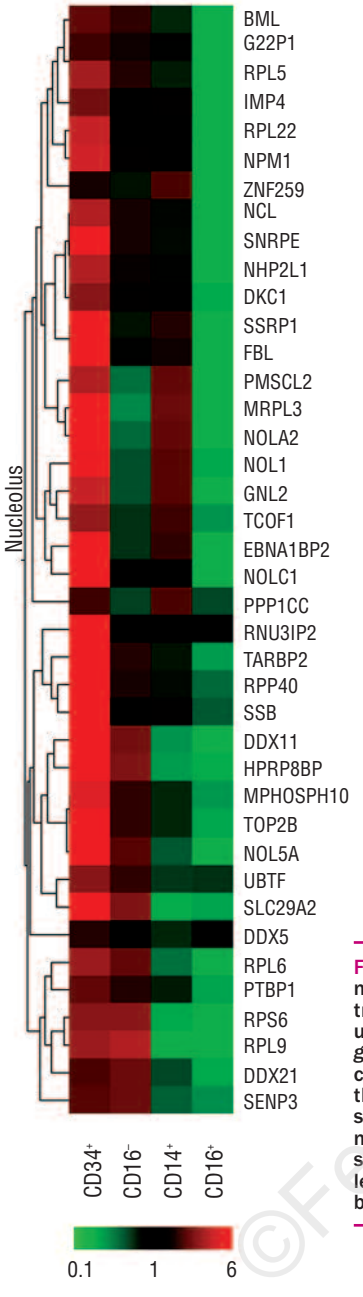

Figure 4. Expression of nucleolar genes. Eisen tree map computed using the GeneSpring *gene* tree and Pearson's correlation equation on the modulated probe sets belonging to the nucleolus category. The signal-based coloring legend is shown at the bottom of the figure.

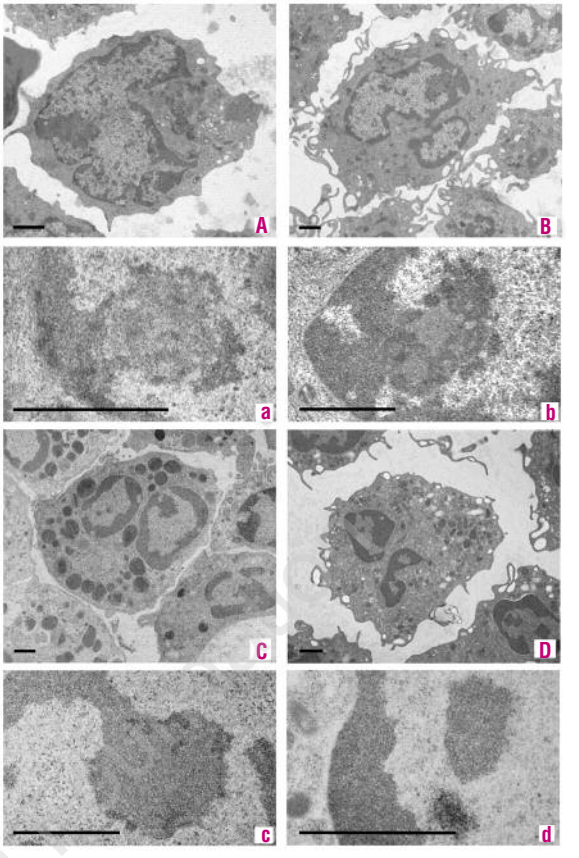

Figure 5. Transmission electron microscopy of CD34$^+$ (A,a), CD14$^+$ (B,b), CD16$^-$ (C,c) and CD16$^+$ (D,d) cells. Large, marginated nucleoli are evident in CD34$^+$, CD14$^+$ and CD16$^-$ cells. At higher magnification (a,b,c,d) the ultrastructure of granular and filamentous components of nucleoli is shown. Nucleolar segregation is frequently observed in CD16$^+$ cells (d). Bar: 1 μm.

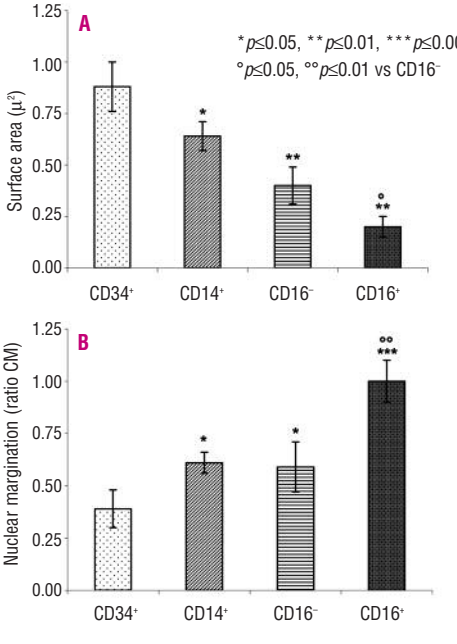

Figure 6. Morphometric analysis. Evaluation of the nucleolar surface (A) and of the ratio of centrally located/marginated nucleoli (C/M) (B) in CD34$^+$, CD14$^+$, CD16$^-$ and CD16$^+$ cells. Significance of data was assessed by unpaired Student's t test (A) and by the χ^2 test (B).

Neutrophils are highly specialized phagocytic cells that circulate in the blood for only a few hours before migrating out of capillaries into the connective tissues or other specific sites, where they survive for only a few days. In contrast, circulating monocytes give rise to a variety of tissue-resident macrophages, as well as to specialized cells such as dendritic cells and osteoclasts. Monocyte-derived macrophages can persist for months or perhaps even years outside the bloodstream, where they can be activated by local signals to resume proliferation. Due to their highly restricted specialized functions, short-lived properties and lack of replication and differentiation ability, neutrophils are commonly considered terminally differentiated (end-stage) cells, while monocytes (macrophages) are not,

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because they are multifunctional pleiotropic cells and maintain some differentiation potential.

Since their discovery, the function of eosinophils has been disputed. Eosinophils are mainly tissue cells, attracted to mucosal sites by several chemotactic factors. They express numerous surface receptors, which make them sensitive to several differentiation, activation and inflammatory signals. Despite often being dismissed as short-lived and relatively limited effector cells regulated by T cells, eosinophils may give diverse contributions to immunity and homeostasis. In some circumstances tissue damage and disease may be the end result, whereas in others, eosinophils may be important contributors to the development and maintenance of normal tissues.[2] Moreover, in recent years, several authors demonstrated that eosinophils, unlike neutrophils, are able to survive at least 12 days in inflammed tissues[27,28] and there contribute to tissue homeostasis and remodeling. For these reasons, despite the fact that eosinophils have so far been considered very similar to neutrophils, it is conceivable that these complex functions require a more flexible cell machinery than that of a terminally differentiated cell.

We, therefore, studied the eosinophil transcriptome profile and compared it with the profiles of neutrophils, monocytes and $CD34^+$/hematopoietic progenitor cells.

Unsupervised analysis of the gene expression profiles showed that the transcriptome profile of eosinophils was hierarchically closer to monocytes than to neutrophils. We then focused on the transcriptome differences between eosinophils, monocytes and neutrophils.

The analysis of biological processes in which the differentially expressed genes were involved demonstrated that, compared to neutrophils, eosinophils and monocytes upregulate genes involved in nucleolus activity and in DNA repair. These results encouraged us to asses the functionality of the nucleolus and the DNA repair machinery in eosinophils. Our data demonstrate that eosinophils maintain the ability to repair both double- and single-strand DNA breaks, while neutrophils lack this capacity. This supports the concept that eosinophils are not terminally differentiated cells; in fact, fully efficient DNA repair machinery is essential for the maintenance of genomic stability of hematopoietic progenitors and precursors, as well as mononuclear phagocytes.[29,30] Moreover, mismatch repair deficiency is associated with a hematopoietic repopulation defect and stem cell exhaustion because of accumulation of genomic instability.[29]

In agreement with this concept we also demonstrated that eosinophils show a nucleolar activity more closely resembling that of monocytes than that of neutrophils. Our results confirmed previous data on the presence and number of nucleoli in hematopoietic progenitors, monocytes and granulocytes[18,31] and provided some missing information on the nucleoli of eosinophils, showing that these cells contain small nucleoli and some ring-shaped nucleoli. The presence of ring-shaped nucleoli has been associated with an immature cell state, which may be stimulated to further transformation,[19] thus supporting the idea that eosinophils may be capable of additional maturation.

Moreover, TEM analysis demonstrated that eosinophils, when compared to neutrophils, are characterized by a higher degree of nucleolar margination, which is an indication of more active protein synthesis.[26] Conversely, nucleolar segregation, related to a decrease of RNA polymerase I activity,[25] is more evident in neutrophils.

Of particular interest is the finding that nucleophosmin (NPM1), a nucleolar protein involved in ribosome biogenesis and in maintaining genome stability,[32] is up-regulated in $CD16^-$ cells as compared to the level in $CD16^+$ cells.

By regulating p53 in response to DNA-damaging stress, NPM1 may provide a survival mechanism which allows the cell to ultimately repair the damage.[33] NPM binds both DNA and RNA, and functions as a histone chaperone during the assembly of new nucleosomes and after DNA lesions have been repaired.[34] NPM1 also plays roles in chromatin remodeling and assembly.[35]

The presence of large, active nucleoli in eosinophils, coupled with the high activity of these cells' DNA repair systems and NPM1 overexpression, may suggest that cosinophils could undergo further maturation or activation, as described for monocytes, whereas neutrophils do not have these properties. In fact, circulating monocytes give rise to a variety of tissue-resident macrophages throughout the body, as well as to specialized cells such as dendritic cells and osteoclasts.[36] Blood monocytes continuously repopulate macrophage or dendritic cell populations to maintain homeostasis and, during inflammation, play critical roles in innate and adaptive immunity.[37]

Similarly, it is plausible that eosinophils maintain nucleolar and DNA repair functions because they play, as previously described, important roles in the development and homeostasis of normal tissues. These functions require active protein synthesis and DNA repair activities, which could provide eosinophils with a longer half-life, as already described for eosinophilic syndromes,[38] and the capacity to affect tissue homeostasis and remodeling.

Collectively, our results strongly suggest that eosinophils, even if considered end-stage granulocytes, are complex multifunctional leukocytes, showing a transcriptome profile and functional properties closer to those of non-terminally differentiated cells such as monocytes.

Authors' Contributions

SS and EB performed the comet and AgNOR assays, RNA staining and contributed to microarray data analysis; RZ, ET performed cell purification and microarray analysis; DQ performed TEM analysis; RM & SF designed the research and wrote the paper.

Conflict of Interest

The authors reported no potential conflicts of interest.

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