Journal of Biological Regulators and Homeostatic Agents

# Reactive phenotypes after acute and chronic NK-cell activation

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**ABSTRACT: Several phenotypic changes have been shown to occur after NK-cell stimulation, involving molecules that have been proved to regulate NK-cell migration into tissues and NK-cell activation and proliferation as well as target cell recognition and killing. Here, we review the reactive phenotypes observed *in vivo* after acute and chronic NK-cell activation. (J Biol Regul Homeost Agents 2004; 18: )**

**KEY WORDS:** *NK-cells, Activation, Phenotype, Flow cytometry*

*Received:*
*Revised:*
*Accepted:*

## Introduction

Most of our knowledge on the modulation of the cellular surface phenotypes associated with NK-cell activation is based mostly on *in vitro* studies. In accordance, it has been shown that activated NK-cells express a number of molecules that are absent in non-activated NK-cells, and that NK-cell activation results in either down-regulation or up-regulation of different receptors constitutively expressed by resting NK-cells, whereas the surface levels of other molecules remain stable (1-13).

In order to define the reactive phenotypes after acute and chronic NK-cell activation *in vivo*, we have analyzed by four-color flow cytometry the immunophenotype of blood $CD56^{+low}$ NK-cells in normal individuals (14), in patients with acute viral infections and in patients with either chronic infections or tumors (15) (Fig. 1). This strategy allowed us to characterize different subsets of normal NK-cells, corresponding to early and late activation-related stages (15), to compare reactive NK-cell phenotypes with those observed on T-cells (16) and to identify abnormal NK-cell leukemia-associated phenotypes (17).

## Normal and reactive NK-Cell phenotypes

### *Normal blood $CD56^{+low}$ NK-cells*

Although a great inter-individual variability is found in normal individuals due to the presence of different NK-cell populations in different maturation and activation states, the majority of the $CD56^{+low}$ NK-cells present in normal blood have a $CD2^{-/+low}$, $CD7^{+high}$, $HLA\text{-}DR^{-}$ and $CD45RO^{-}$ phenotype (14). Most of these cells are $CD11b^{+}/CD38^{+}$ and homogeneously express high levels of CD11a and CD45RA, whereas CD11c and CD57 are heterogeneously expressed in a variable fraction of $CD56^{+low}$ cells.

### *$CD56^{+low}$ NK-cells from patients with acute viral infection*

Recently activated $CD56^{+low}$ NK-cells are present at high frequency in the blood of patients with acute viral infection (15). They have a pattern of expression of CD2 and CD7 similar to that observed in resting mature $CD56^{+low}$ NK-cells, except that a higher percentage of $CD2^{+}$ NK-cells and a slightly higher intensity of CD7 expression are found. $CD2^{+low}/CD7^{+high}$ CD56+low NK-cells probably originate from $CD2^{-}/CD7^{+high}$ CD56+low NK-cells upon stimulation, through *de novo* expression of CD2 molecules on the cell surface. Recently activated $CD56^{+low}$ NK-cells typically show strong reactivity for CD38 and express high levels of HLA-DR. At that phase, there is also down-regulation of CD45RA, a phenomenon that is transiently accompanied by co-expression of CD45RO in variable proportions of cells. CD11a levels are increased, whereas expression of CD11b is decreased. Moreover, these $CD56^{+low}$ NK-cells express dimly and heterogeneously CD11c while they are either negative or dimly positive for CD57.

### *$CD56^{+low}$ NK-cells from patients with chronic infections and tumors*

Chronically activated $CD56^{+low}$ NK-cells shown unique phenotypic features (15), including up-regulation of CD2, which becomes homogeneous ($CD2^{+HIGH}$), and down-regulation of CD7, which

0393-974X/331-04 $15.00

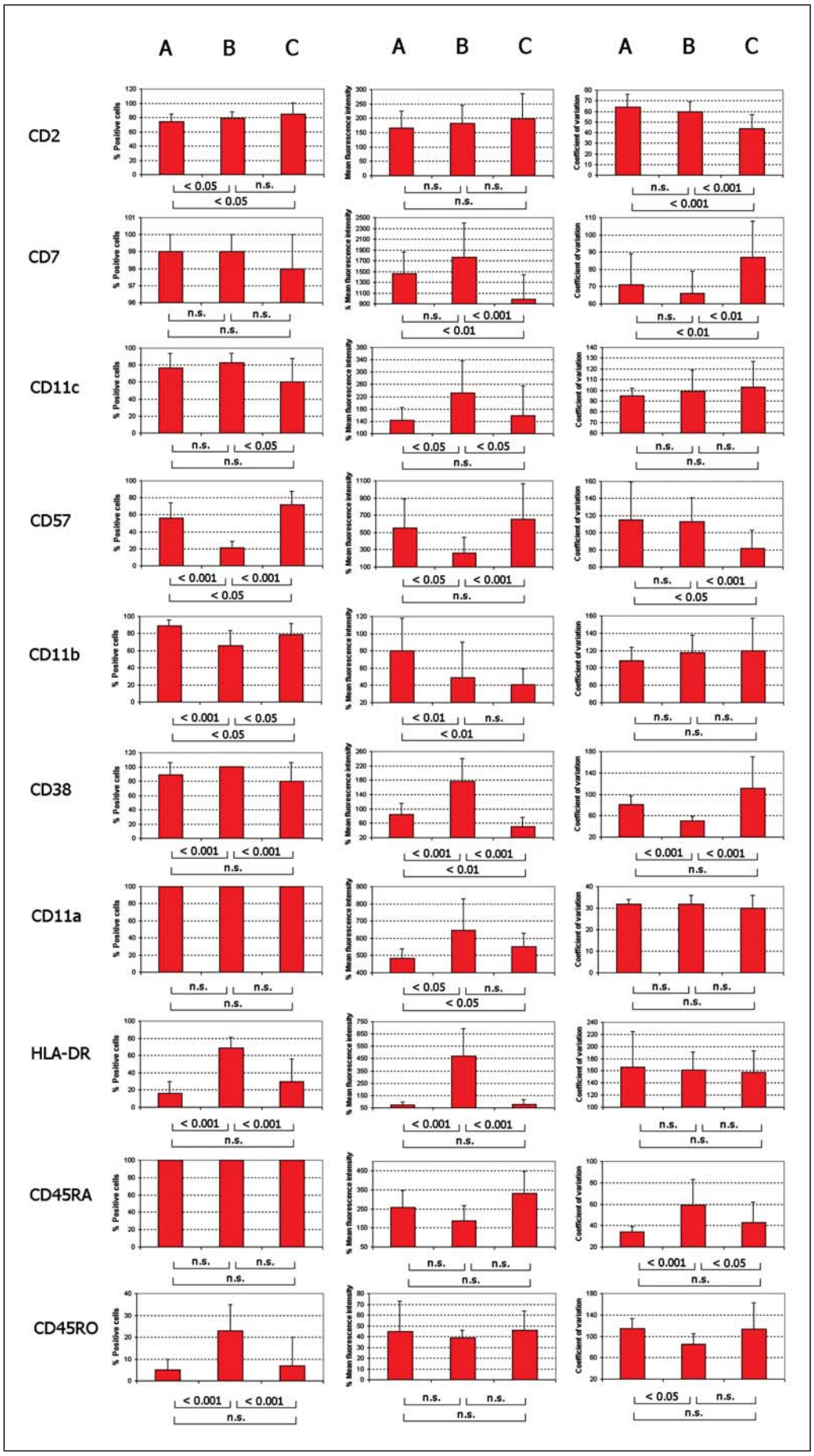


**Fig. 1 - Immunophenotypic analysis of blood CD56$^{+low}$ NK-cells in normal individuals (A), patients with acute viral infection (B) and patients with NK-cell lymphocytosis secondary to infections or tumors (C). APC-conjugated anti-CD3 and PE-Cy5- conjugated anti-CD56 were used in combination with the following pairs of FITC- / PE- conjugated monoclonal antibodies directed against T- and NK-associated antigens: CD2/CD7, CD57/ CD11c, CD38/ CD11b, CD11a/ HLA-DR, CD45RA/ CD45RO. Results are expressed as percentage of positive cells, mean fluorescence intensity (arbitrary relative linear units scaled from 0 to 10,000) and coefficient of variation. Bars represent the mean values and the vertical lines represent the standard deviation. The statistical significance of differences observed between groups was evaluated using the Mann-Whitney U-test. P-values less than 0.05 were considered to be associated with statistical significance. ns, not statistically significant.**

becomes heterogeneous ($CD7^{-/+low}$). In contrast to recently activated CD56+low NK-cells, chronically activated $CD56^{+low}$ NK-cells are $CD38^{-/+low}$ and $CD11b^{-/+low}$ and express high and homogeneous levels of CD57, while CD11c expression is dim and heterogeneous. At this stage, most NK-cells have already reverted into their original HLA-$DR^{-}$/$CD45RA^{+}$/$CD45RO^{-}$ phenotype, although a variable proportion of HLA-$DR^{+}$ and/or $CD45RO^{+}$ NK-cells can still be found in some individuals.

## Differences and similarities between reactive NK-cell and T-cell phenotypes

Patients with infectious mononucleosis show a massive expansion of $CD8^{+}$ TCRalpha/$beta^{+}$ T-cells, the majority of which display an immunophenotype compatible with recent T-cell activation (16): $CD2^{+high}$, $CD7^{-/+low}$, $CD11a^{+high}$, $CD38^{+high}$, HLA-$DR^{+}$, $CD45RO^{+high}$, $CD45RA^{-/+low}$, $CD11b^{-/+low}$, $CD11c^{-/+low}$, $CD57^{-}$. Additionally, the levels of CD3, CD5, CD28 and CD62L molecules are decreased compared to those found in normal individuals. Late-activation antigens, such as CD57, were found in small proportions of $CD8^{+}$ TCRalpha/$beta^{+}$ T-cells. Increased numbers of activated $CD4^{+}$ TCRalpha/$beta^{+}$ and TCRgamma/$delta^{+}$ T-cells are also observed in some patients. Evidence for activation of $CD4^{+}$ TCRalpha/$beta^{+}$ and TCRgamma/$delta^{+}$ T-cells rely on changes similar to those described for $CD8^{+}$ TCRalpha/$beta^{+}$ T-cells.

Curiously, most of the immunophenotypic changes that occur after NK-cell activation are similar to that observed on T-cells, although different kinetics were observed in some markers. For instance up-regulation of CD11c, CD38 and CD45RO are transiently observed in early T- and NK-cell activation stages, as do *de novo* expression of HLA-DR molecules; modulation of CD2 and CD7 expression occurs in both cases, consisting in up-regulation of CD2 and down regulation of CD7 molecules, which seems to take place at a latter stage during NK-cell activation, as compared to that occurring on T-cells; down-regulation of CD7 expression is probably preceded by a transient up-regulation of this molecule in both cases; CD57 behaves as a late activation marker for both T- and NK-cells.

## Aberrant NK-Cell phenotypes

We have recently reported on the immunophenotypic features of a particular type of chronic NK-cell leukemia, whose NK-cells were either $CD56^{-}$ or expressed very low levels of CD56 ($CD56^{-/+low}$ NK-cells), in the context of an aberrant activation-related mature phenotype (17). $CD56^{-/+low}$ NK-cells display several unusual immunophenotypic features. Accordingly, besides being $CD56^{-/+low}$, they are $CD11b^{-/+low}$ (heterogeneous), $CD7^{-/+low}$ (heterogeneous), $CD2^{+}$ (homogeneous), CD11c+high (homogeneous), $CD38^{-/+low}$ (heterogeneous), $CD94^{+high}$ (heterogeneous) and HLA-$DR^{+}$.

Some of the immunophenotypic differences found between normal and $CD56^{-/+dim}$ NK-cells – i.e. over-expression of CD11c and HLA-DR and down-regulation of CD45RA accompanied in some cases by co-expression of CD45RO in a variable fraction of NK-cells - do in fact reproduce the immunophenotypic changes that typically occur on recently activated $CD56^{+low}$ NK-cells, whereas other – i.e. increased CD2 expression and decreased and heterogeneous expression of CD7, CD11b and CD38 – are similar to those typically observed in conditions of chronic NK-cell stimulation. In spite of these similarities between $CD56^{-/+low}$ NK-cells and either recently- or chronically-activated $CD56^{+low}$ NK-cells, major differences are observed in the pattern of expression of some markers. Accordingly, $CD56^{-/+low}$ NK-cells have higher and more homogenous expression of CD11c, greater percentages of $CD57^{+}$ cells and lower levels of HLA-DR as compared to recently activated NK-cells. Additionally, CD2 expression is more homogeneous, down-regulation of CD7 and CD11b is more pronounced and CD7 expression is even more heterogeneous on $CD56^{-/+low}$ NK-cells than on chronically activated $CD56^{+low}$ NK-cells. Moreover, disagreement between down-regulation of CD11b and CD38 expression, consisting on a $CD11b^{-}$/$CD38^{+}$ phenotype, is frequently found in $CD56^{-/+dim}$ NK-cells but not on “chronically” activated $CD56^{+low}$ NK-cells.

Such aberrant activation-related immunophenotype was the very first argument supporting the monoclonal nature of these $CD56^{-/+low}$ NK-cells, which was subsequently confirmed in female patients using the human androgen receptor gene polymerase chain reaction -based assay.

## Concluding remarks

The majority of changes that have been documented upon NK-cell activation *in vitro* are also reproducible *in vivo* and define phenotypic patterns associated to the early/acute- and late/chronic-phases of NK-cell activation.

Early activation-related NK-cell phenotypes are found in patients with acute viral infection whereas in only a minor proportion of patients with chronic infections and tumors and normal healthy individuals. Similarly, chronically-activated NK-cells are over-represented in patients with NK-cell lymphocytosis associated to conditions in which the underlying stimuli persist for a long time, although they could also be found at lower numbers in a few percentage of patients with acute infections as well as in normal individuals.

Finally, by understanding the sequence of the immunophenotypic changes occurring after NK-cell

activation, we are able to identify aberrant immunophenotypic patterns and to distinguish reactive NK-cell lymphocytosis from NK-cell leukemia cases.

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