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# Human Leukocyte Antigen Class II in Stimulated Polymorphonuclear Neutrophils

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## 1. Introduction

### 1.1 Polymorphonuclear neutrophils (PMN)

Polymorphonuclear neutrophils (PMN) possess a very short half-life in the circulation because they constitutively undergo apoptosis (Gasmi et al., 1996; Stringer et al., 1996; Moulding et al., 1998). Under certain conditions PMN play an important role in the effectors arm of host immune defense through the clearance of immune complexes, the phagocytosis of opsonized particles, and the release of inflammatory mediators (Shen et al., 1987; Petroni et al., 1988; Lloyd et al., 1992). During the recent years the image of PMN has changed considerably. Traditionally considered to be the first line defense against bacterial infection. It became increasingly clear that PMN also participate in chronic inflammatory disease and regulation of the immune response when appropriately activated (Iking-Konert et al., 2002). Persistent *Staphylococcus aureus*-mediated local infection induces the local activation and transdifferentiation of PMN to cells with dendritic-like characteristics (Wagner et al., 2006).

### 1.2 Interleukin (IL)

Interleukin is involved in processes of cell activation, cell differentiation, proliferation and cell to cell interactions. Each IL acts on a specific group of cells that express the correct receptor for the IL. The same IL can be produced by different cell types and an individual IL may act on different cell types, eliciting variable biological responses depending on the particular cell and its environment (Bhandari, 2002).

#### 1.2.1 Interleukin (IL)-2

IL-2 has been considered to be a lymphocyte-activating and growth-promoting factor, and has been widely studied on T cells and NK cells (Waldmann, 1989). Monocytes have been reported to express IL-2R $\alpha$  and to be activated by IL-2 for tumoricidal activity (Espinoza-Delgado et al., 1990). Thus far, PMN have not been studied for their interaction with IL-2, and their possession of IL-2R is unknown. The direct effect of IL-2 on PMN, especially the mechanisms involved in the activation of PMN, is unknown, although the ability of other immune cells to respond to IL-2 is well studied. Preliminary studies have shown that PMN have the capacity to respond to IL-2 with increased antifungal activity (Djeu et al., 1993).

More importantly it have identified that PMN express surface receptors for IL-2, but only IL-2R $\beta$  and not IL-2R $\alpha$  is present (Djeu et al., 1993).

### 1.2.2 Interleukin (IL)-4

IL-4 production has been found to occur in thymocytes, mature T-cells, certain malignant T-cells, mast-cells and basophiles and occasionally, in transformed B-cells (Holter, 1997). It has an effect on B-cells, T-cells, monocytes, mast-cells, endothelial cells, and fibroblasts (Paul, 1991). Directly and/or indirectly, IL-2 has a prominent role in the regulation of IL-4 producing cells (Holter, 1997). IL-4 binds to a high-affinity cell-surface receptor (IL-4R) to exert its effects (Idzerda et al., 1990). It promotes the growth and differentiation of activated human B-lymphocytes and shares many biological functions with IL-13 (Aversa et al., 1993).

### 1.2.3 Interleukin (IL)-15

IL-15 is a pleiotropic cytokine which shares biological activities with IL-2. IL-15 uses both  $\beta$ - and  $\gamma$ -chains of the IL-2 receptor for binding and signaling. The IL-15 receptor (IL-15R) complex also includes a specific  $\alpha$  subunit (IL-15R $\alpha$ ), distinct from the IL-2R $\alpha$  chain. IL-15R is expressed on various cells of the immune response, including T- and B-cells, NK cells and more recently, peripheral blood neutrophils (Girard et al., 1999).

IL-15 plays an important role in both innate and adaptive immunity. It induces T-cell proliferation and cytokine production, stimulates the locomotion and chemotaxis of T-cells and delays its apoptosis. IL-15 has been shown to stimulate the growth and cytotoxicity of NK cells and to induce antibody-dependent cell-mediated cytotoxicity. It was also demonstrated that this cytokine induces macrophages function and B-cell proliferation (Girard et al., 1999). Recent investigation has shown that IL-15 potentiated several antimicrobial functions of normal PMN involved in the innate immune response against invading pathogens (Casatella and McDonald, 2000). IL-15 was observed to enhance phagocytosis, NF- $\kappa$ B activation, and IL-8 production and to delay apoptosis of these cells. In addition, IL-15 has been shown to prime the metabolic burst of PMN in response to N-formyl-methionyl-eucyl-phenylalanine (fMLP) (Casatella and McDonald, 2000; Girard et al., 1996; McDonald et al., 1998; Mastroianni et al., 2000).

The main source of IL-15 are macrophages / monocytes and lymphocytes as well as neutrophils (Casatella and McDonald, 2000; Muro and Taha, 2001; Zissel and Baumer, 2000).

## 1.3 Human leukocyte antigen (HLA) class II

For surface molecules, there are several reports that PMN from a variety of species can express HLA class II (Vachier et al., 1999; Radsak et al., 2000; Iking-Konert et al., 2001a; Iking-Konert et al., 2002; Tyler et al., 2006). Under certain stimulation murine neutrophils present Class II restricted antigen (Shauna et al., 2008). Coexpression of HLA class II would potentially endow PMN with the capacity to influence the adaptive immune system through antigen presentation. Previous investigations with mouse, human, and goat PMN defined conditions whereby HLA class II expression was observed following stimulation with IFN- $\gamma$ , granulocyte macrophage-colony stimulating factor (GM-CSF), or IL-3 (Gosselin et al., 1993; Smith et al., 1995; Mudzinski et al., 1995; Reinisch et al., 1996) or in human patients,

with Wegener's granulomatosis (Haensch et al., 1999; Iking-Konert et al., 2001b). In some cases, PMN expression of HLA II was reported as constitutive (Okuda et al., 1979; Vachieri et al., 1999), and human PMN were also shown to function as accessory cells for primed T-cell activation with protein antigens and superantigens (Okuda et al., 1980; Fanger et al., 1997; Iking-Konert et al., 2001a; Tyler et al., 2006). Bovine PMN expressed detectable levels of HLA class II on their surface, only when cocultured with peripheral blood mononuclear cells (PBMC). This observation suggested that PMN may up-regulate endogenous HLA class II expression or passively acquire HLA class II protein from other leukocytes.

To better understand the role of IL-2 in the activation of PMN and to explore the possibility that the activation of PMN by IL-2 may reflect the interaction between T-cells and PMN. These findings provide useful insight for understanding some mechanisms of T-cell and PMN interactions and the possible role of PMN in IL-2 immunotherapy. There is evidence that HLA class II-positive PMN are able to present the superantigen staphylococcus enterotoxin E (SEE) to T-cells in an HLA class II-dependent manner (Fanger et al., 1997). Moreover, PMN pulsed with peptide was shown to activate antigen-specific memory T-cells (Reali et al., 1996). In addition, PMN stimulated with GM-CSF and IFN- $\gamma$  has the capacity to present antigen to naive T-cells (Fanger et al., 1997). Those PMN with antigen-presenting capacity are thought to be a relatively mature population (Mudzinski et al., 1995). There is a large production of proinflammatory cytokines from both T-cells and monocytes following superantigen activation: T-cells produce IL-1 $\beta$ , IFN- $\gamma$ , TNF- $\alpha$ , and IL-2 (Jupin et al., 1988; See et al., 1992a; Lagoo et al., 1994). While monocytes produce both IL-1 $\beta$  and IFN- $\gamma$  (See and Chow 1992; See et al., 1992; Trede et al., 1994). It has shown that human PMN can be induced to express HLA class II molecules both *in vitro* and *in vivo*. Specifically, *in vitro* incubation of human PMN from healthy donors with either GM-CSF, IFN- $\gamma$ , or IL-3 resulted in low-level expression of HLA-DR (Gosselin et al., 1993; Smith et al., 1995). In rheumatoid arthritis, there is an evidence for T-cells activation, where PMN act as dendritic-like cells at the site of inflammation (Iking-Konert et al., 2005).

#### 1.4 Aim of the study

The aim of this study was to insure that IL-2, IL-4 or IL-15 stimulated PMN might be involved in T-cell proliferation by acquiring HLA class II antigens.

## 2. Materials and methods

### 2.1 Immunocytochemistry

Blood was taken by venous puncture using 7.5 ml heparin-coated tubes (Sarstedt; Nümbrecht, Germany) and was analyzed within 2 hours

The IL-2R of PMN could be detected by immunofluorescence. Freshly isolated PMN by two hypotonic/hypertonic lyses steps with 0.2%/1.6% saline were fixed on slides (2 X 10<sup>5</sup> cells / slide) by a cytoSpin 4 centrifuge (Shandon; Frankfurt, Germany) and ice-cold methanol. Cells were incubated with 5% goat serum (Sigma; Saint Louis, MO, USA) in PBS followed by 2  $\mu$ g anti-CD25-FITC (Becton Dickinson; San Jose, USA), 2  $\mu$ g anti-CD122-FITC (Serotec; Oxford, UK). Anti-mouse IgG-FITC and anti-CD66-FITC (Immunotech. Marseille, France) were used as positive and negative control respectively. The slides were examined by confocal laser microscopy (Leica, Bensheim, Germany) using Windows TC as software.

## 2.2 Cytofluorometry

IL-2R was detected on PMN by direct and indirect extracellular fluorescence-activated cell sorter (FACS). In direct sets of experiments cells in whole blood were stained with 2 $\mu$ g anti-CD122-FITC and 2 $\mu$ g anti-CD25 (Serotec; Oxford, UK) as a markers for IL-2R $\beta$  chain and IL-2R $\alpha$  chain. Primary an unlabeled antibody for IL-2R $\beta$  chain was added to cells before staining with anti-CD122-FITC. Anti-mouse IgG-FITC and anti-CD66-FITC (Immunotech.; Marseille, France) were used as a negative and a positive control, respectively.

For HLA class II detection cells in whole blood were double labeled with 2 $\mu$ g anti-CD66b-FITC (Immunotech.; Marseille, France) as a PMN marker and 2 $\mu$ g PE-labeled antibodies to HLA DP-DQ-DR (Serotec; Oxford, UK), respectively, using standard procedures. They were analyzed by FACSCalibur and CellQuest software (Becton-Dickinson, Heidelberg, Germany). Results are expressed as percentage of positive cells in the respective gate or quadrant.

## 2.3 Cells purification and cultivation

For co-culture, cells were isolated by PolymorphPrep® (Nycomed; Oslo, Norway). PMN, Monocytes and T-cells fraction was further purified by adsorption to CD15, CD14 and CD3 beads (Miltenyi Biotech; Bergisch Gladbach, Germany), respectively, by magnetic cell separation using the devices supplied by Milteny Biotech (Bergisch-Gladbach, Germany).

Highly purified PMN (1  $\times$  10<sup>6</sup> /ml) were cultivated in AIM V (Gibco BRL; Paisley, Scotland)) with 2.5% autologous normal human serum, NHS (inactivated at 56°C for 30 min.). T-cells and monocytes were cultured in RPMI 1640 (Gibco BRL; Paisley, Schottland) supplemented with 10% FCS (PAN Biotech GmbH; Aidenbach, Germany), 100U/ml penicillin/streptomycin (Gibco BRL; Paisley, Schottland), 2mM L-glutamine (Gibco BRL; Paisley, Schottland)), and 10mM HEPES (Gibco BRL; Paisley, Scotland). All the three types of cells were incubated at 37°C and 5 % CO<sub>2</sub> for the times indicated.

## 2.4 Stimulation of PMN

Highly purified PMN were placed into 24-well plate (Nunc™; Roskilde, Danmark)), 2ml/well, and incubated in the presence or absence of 10ng/ml IL-2 (Sigma; St Louis, MO, USA)) for about 48 hours at 37°C with 5% CO<sub>2</sub>.

## 2.5 RNA isolation and reverse transcription-polymerase chain reaction (RT-PCR)

Total cellular RNA was isolated using the RNeasy kit from Qiagen (Hilden, Germany). For RT, 2 mg of RNA was incubated with 50 pmol random primer, 1 U RNase inhibitor, 10 pmol dNTP and 20 U Moloney Murine Leukemia Virus (MMuLV) reverse transcriptase (all purchased from Boehringer Mannheim, Germany) for 60 min at 37u, followed by 15 min at 94u. PCR for HLA class II was carried out in a Perkin-Elmer (UÈ berlingen, Germany) thermocycler as follows: 10 pmol dNTP was added to 5 ml of cDNA, followed by 50 pmol of the primers and 1 U Taq DNA polymerase in 2 mM MgCl<sub>2</sub>. After preheating to 94u for 10 min, 30 cycles for HLA class II were performed (30 seconds at 94u, 30 seconds at 60u, 60 seconds at 72u) followed by a final extension step at 72u for 10 min. The HLA-DR primer was used: sense: 5k-CGGATCCTTCGTGTCCCCAC-3k; antisense: 5k-

CTCCCCAACCCCGTAGTTGTGTCTGCA-3k, amplifying a 270-bp fragment (Nadler et al., 1994). This primer were synthesized by ARK Scientific Biosystems (Darmstadt, Germany). The PCR products were analyzed by gel electrophoresis (1.5% agarose) and staining with Sybr green (Molecular Probes; Leiden, the Netherlands). As a size marker, DNA molecular weight marker VI (Boehringer: Mannheim, Germany) was used. For sequencing, the PCR product was extracted by using the QIAquick gel extraction kit (Qiagen; Hilden, Germany); sequencing was performed using the ABI PRISM2 Big Dye2 Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems; Warrington, UK). Data were measured and analyzed by Fluorescent Image Analyzer (FLA)-2000 (Becton Dickinson, San Jose, USA).

## 2.6 Coculture expreiments

Unstimulated and stimulated PMN ( $1 \times 10^3$ ) or monocytes ( $1 \times 10^3$ ) in 100 $\mu$ l were added per well of a 96-well concave-bottom plate (Greiner; Nuertingen, Germany). Then,  $1 \times 10^4$  T cells (100 $\mu$ l) were added to each well together with 25ng *Staphylococcal aureus* Enterotoxins A (Sigma; München, Germany). The effect of IL-2 stimulation was blocked by ant-IL-2R (Serotec; Oxford, UK). After coincubation for 4 days at 37°C with 5% CO<sub>2</sub>, proliferation was tested by adding 1 mCi of <sup>3</sup>H-thymidine (Amer-sham Life Science; Braunschweig, Germany) for 6-8 hours [<sup>3</sup>H]TdR incorporation into DNA was measured and expressed as counts per minute (cpm). The values represent the mean $\pm$ SD of 6-12 parallel wells.

## 2.7 Statistical analysis

Data were analyzed by student's t test. And a P value of 0.05 was a considered as the limit of significance.

## 3. Results

### 3.1 Visualization of the IL-2R $\beta$ chain by immunocytochemistry

Isolated PMN by a hypotonic solution were examined by confocal laser microscopy. The IL-2R $\alpha$  chain was not detectable (Fig. 1c). However the IL-2R $\beta$  chain was seen in all cells (Fig. 2c). Mouse IgG-FITC was used as a negative control (Fig. 1a and 2a), while CD66b-FITC was used as a positive control (Fig. 1b and 2b), respectively.

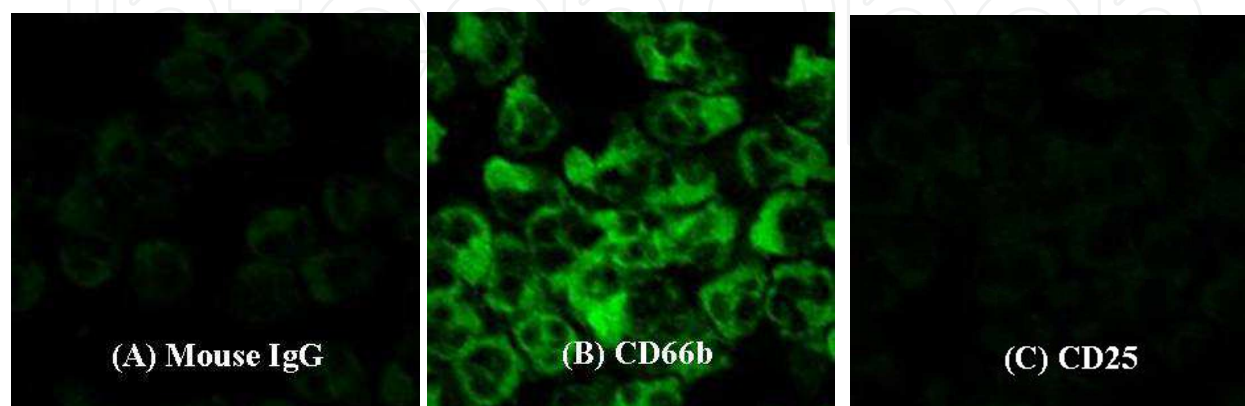


Fig. 1. Detection of IL-2R $\alpha$  chain on PMN: The negative and positive controls were in panels A and B, respectively, while the panel C showed the CD25 (magnification: 1 X 400).

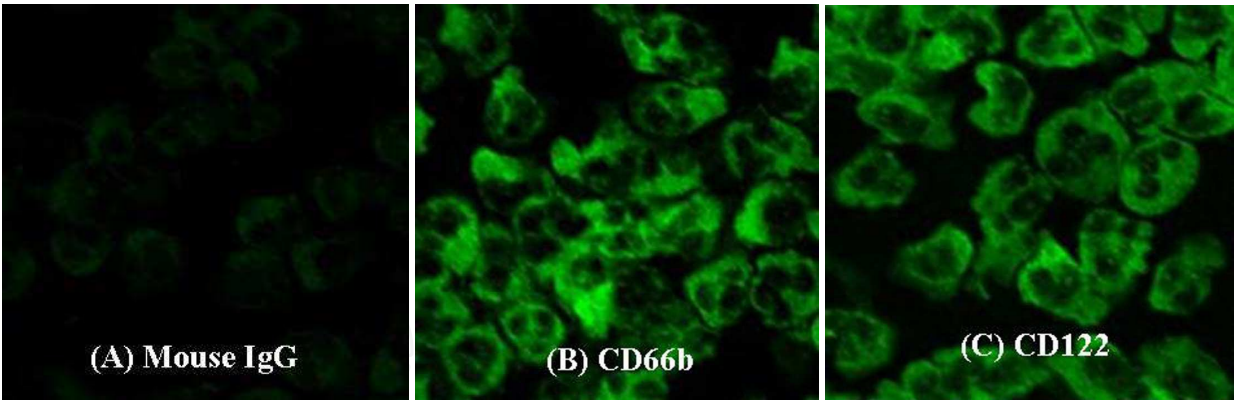


Fig. 2. Detection of IL-2R $\beta$  chain on PMN: The negative and positive controls were in panels A and B, respectively, while the panel C showed the CD122 (magnification: 1 X400).

3.2 Direct immunofluorescence analysis of IL-2R

In order to investigate constitutive expression of IL-2R, we tested the presence of the two IL-2R chains (IL-2 $\alpha$  and IL-2 $\beta$ ) on the PMN cell surface by FACS. In line with previous data by others, the  $\alpha$  chain (CD25) could not be detected (< 1 % positive cells), while PMN expressed CD122, though rather small amounts (Fig. 3).

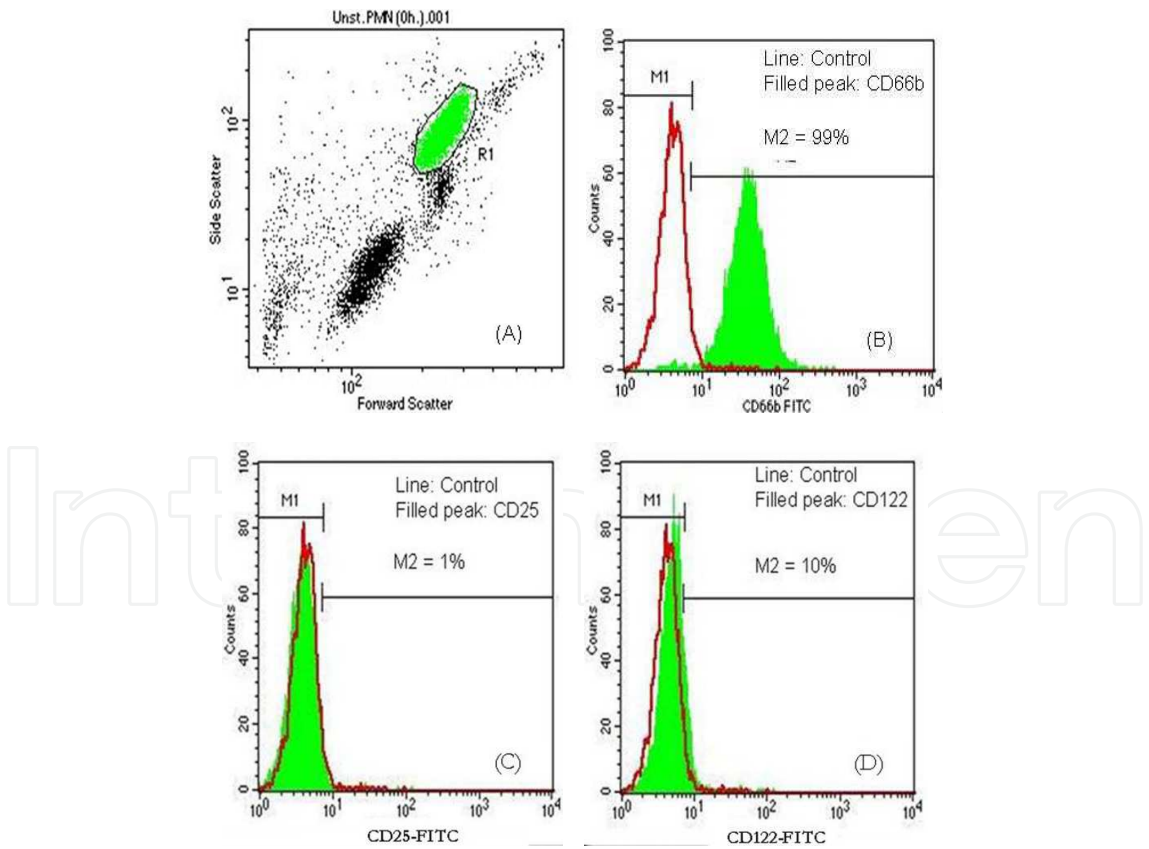


Fig. 3. Detection of IL-2R on PMN: (A) Forward-scatter (FSC) versus side-scatter (SSC) of whole blood flow cytometry showed different populations of leukocytes. R1 was set for PMN identified by expression of CD66b (B) filled peak. PMN expressed only IL-2R $\beta$  chain (D), while IL-2R $\alpha$  chain was absent (C) (filled peaks: antibody; line isotype control).

When comparing cells of different donors, the mean fluorescence intensity varied (a summary of 6 donors is shown in Table 1).

No. of tested donors	Mean fluorescence intensity
1-	10
2-	22
3-	16
4-	14
5-	32
6-	25

Table 1. Expression IL-2Rβ chain (CD122) on PMN of different donors.

3.3 Indirect immunofluorescence for the IL-2Rβ chain

By indirect labeling, using first an unlabeled antibody to IL-2Rβ, followed by a FITC-labeled antibody to mouse IgG, the signal for the IL-2Rβ chain could be amplified. Of note is that nearly all cells were positive (Fig. 4).

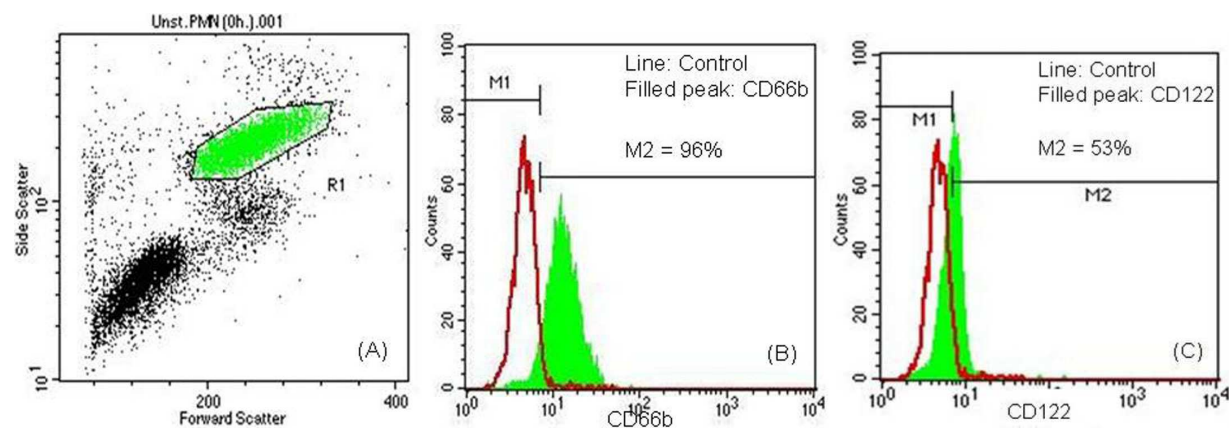


Fig. 4. Indirect extracellular detection of IL-2Rβ chain: (A) the forward-side scatter analysis of whole blood flow cytometry showed different populations of leukocytes. PMN were identified in R1. (B) M2 is the percentage of CD66b-positive cells (filled peak). (C) PMN expressed IL-2Rβ chain as detected by anti-CD122 (filled peak); the line is the negative control using mouse IgG in place of anti-CD122.

3.4 In vitro expression of HLA class II on PMN stimulated with IL-2

To test effects of IL-2 on HLA class II expression, the induction of HLA class II in highly purified PMN 48 hours cultured with IL-2 increased recording 20% (Fig. 5c). Freshly isolated PMN had 2% (Fig. 5a). PMN cultured with medium alone for 48 hours had also small amount (4%) of HLA class II (Fig. 5b).

3.5 Effect of IL-2 on HLA class II gene up-regulation on PMN

In another set of experiments, highly purified PMN exposed to IL-2 for various hours showed an increase in HLA class II specific RNA. This was tested by RT-PCR. When the

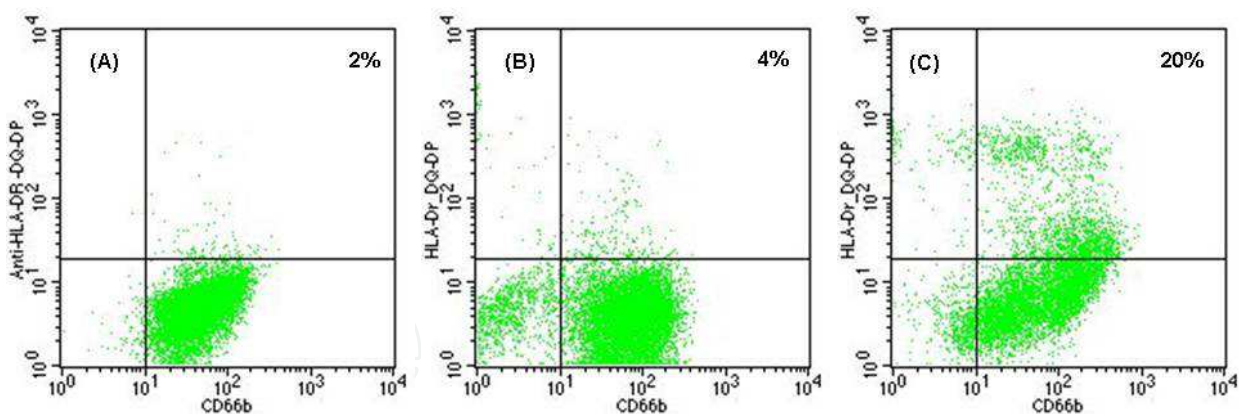


Fig. 5. Direct flow cytometry of the HLA class II induction in highly purified PMN: A) Unstimulated PMN (0 hour). B) Unstimulated PMN (48 hours). C) Stimulated PMN with IL-2 (48 hours).

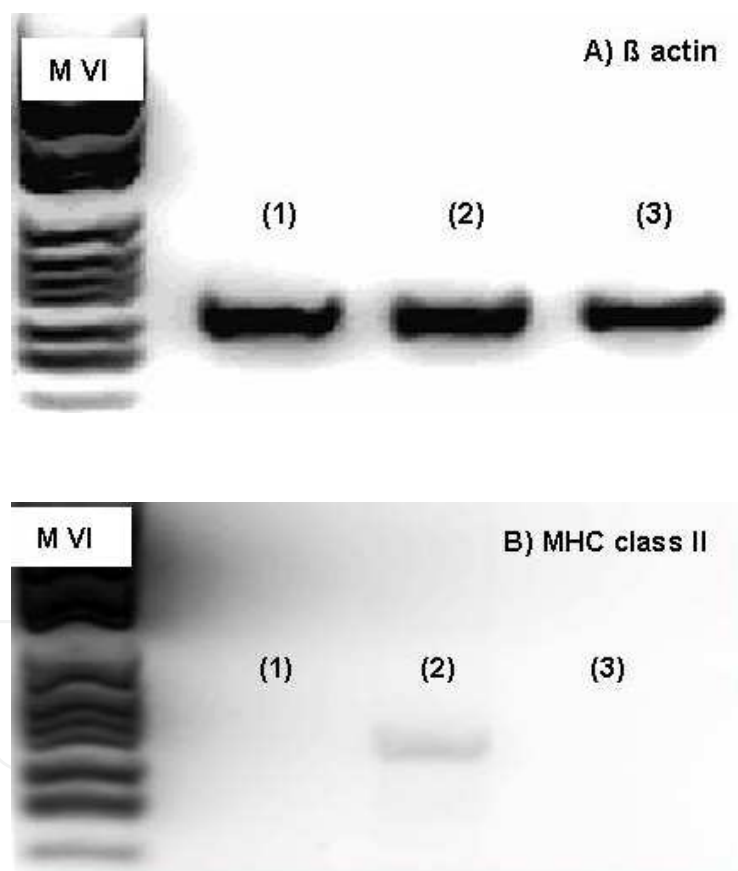


Fig. 6. RT-PCR detection of HLA class II mRNA in PMN activated with IL-2: PMN were incubated with IL-2 for 6 hours. The M VI was the used marker (154-2176 bp). Freshly isolated PMN were in Lane (1). Lane (2) showed the stimulated PMN after 6 hours, while the unstimulated PMN after 6 hours was in lane (3).

values were corrected for the input of the PCR-product by using  $\beta$ -actin as a housekeeping gene (Fig. 6a), it was seen that HLA class II RNA increased only when PMN were cultivated for 6 hours. The increase was more pronounced in the presence of IL-2 (Fig. 6b).

3.6 *In vitro* expression of HLA class II on PMN stimulated with IL-4

The majority of healthy donors PMN expressed CD66b (Fig. 7a, b & c). In the first set of experiments the effect of IL-4 on the expression of HLA class II was tested, where we found that PMN on whole blood showed expression of HLA class II recording 7.77% (Fig.7c) by using IL-4, as stimulators. In contrast, fresh and unstimulated cells cultured with medium only counting 1.05% (Fig.7a) and 2.71 % (Fig.7b), respectively.

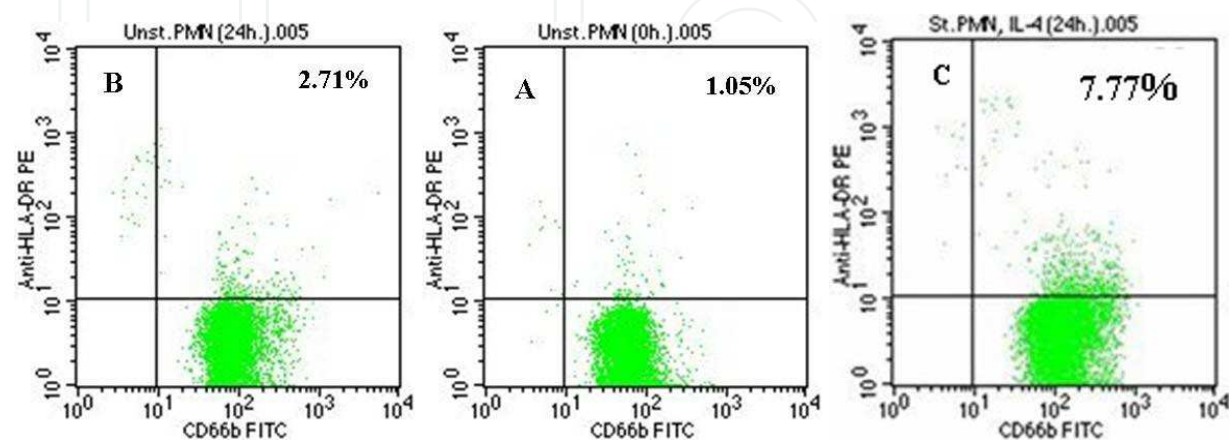


Fig. 7. Direct flow cytometry of the HLA Class II induction in whole blood PMN. A) Unstimulated PMN (0 hour). B) Unstimulated PMN (24 hours). C) Stimulated PMN with IL-4 (24 hours).

3.7 *In vitro* expression of HLA class II on PMN stimulated with IL-15

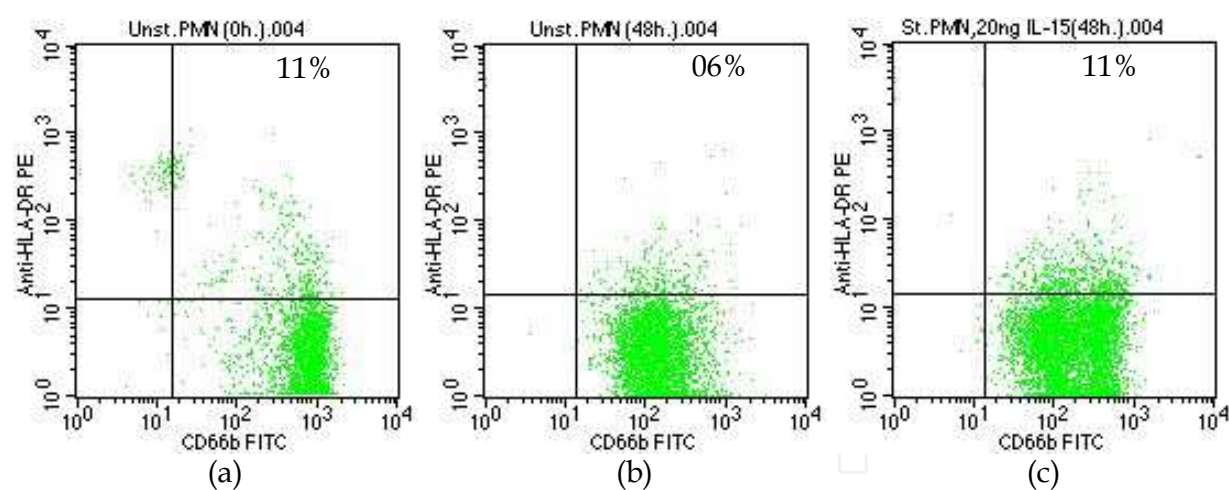


Fig. 8. Flow cytometric analysis of HLA class II expression on PMN treated with IL-15 (20ng / ml). PMN in whole blood were labeled with CD66b-FITC as a specific marker for PMN and HLA class II-PE. (A) Fresh PMN. (B) PMN cultured for 48hrs in medium. (C) PMN cultured for 48hrs in medium with IL-15.

To test effects of IL-15 on HLA class II expression, in a first set of experiments the induction of HLA class II in PMN 24hrs co-cultured with IL-15 was very minor. Although, after 48hrs this induction increased recording 11% (Fig. 8c). Freshly PMN had 11% also (Fig. 8a). while PMN cultured with medium alone had smaller amount (06%) of HLA class II (Fig. 8b).

3.8 Interaction of HLA class II positive PMN with peripheral T-cells

The question was addressed, whether PMN stimulated to express HLA class II antigen, would be able to induce T-cell proliferation. For these experiments highly purified PMN were cultivated with IL-2 for 24 hours and then cocultivated with highly purified isolated T-cells in the absence or presence of *Staphylococcus* enterotoxin A (SEA), a well-known superantigen.

It was imperative to rule out the participation of professional antigen presenting cells, such as dendritic cell, monocytes or B-cells. Therefore great care was taken for purification of the cells, which was controlled cytofluorometry. Using this method, less than 1% contaminating cells could be detected in either the PMN or the T-cell preparation.

In a first set of experiments PMN in various numbers and for comparison monocytes were cocultivated with the T-cells and SEA and proliferation of T-cells was measured after 4 days. The experiment showed that about 10 times more PMN than monocytes were required to achieve comparable proliferation and that 100 monocytes were barely sufficient to induce T-cell proliferation above controls (T-cells alone or T-cells with SEA without PMN or monocytes, respectively) (Fig. 9).

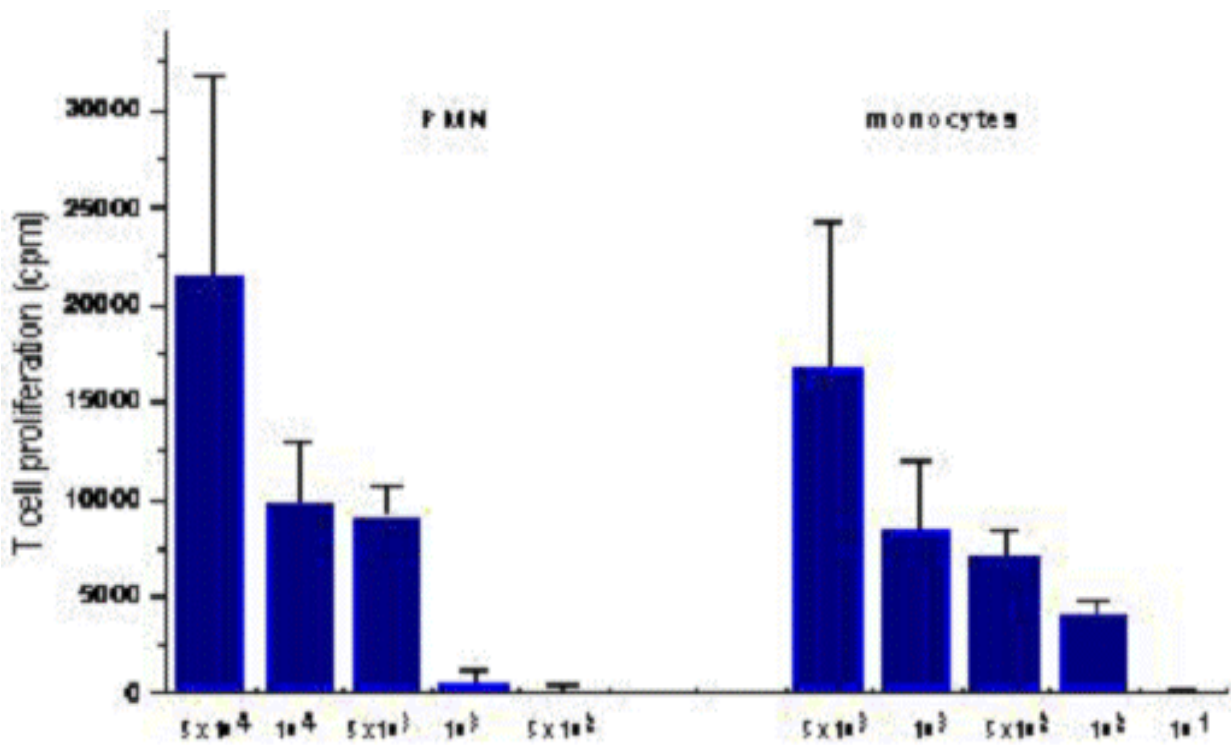


Fig. 9. Cells were cocultured with PMN or monocytes in the numbers indicated and in the presence of SEA. After 4 days proliferation of T-cells was measured by incorporation of  $[^3\text{H}]$ -thymidine, where proliferation was seen with at least 100 monocytes or 1000 PMN.

Based on the initial experiments, in the following 1000 PMN were used, because this would rule out participation of monocytes assuming a 1% contamination.

We could show that the T-cell proliferation was dependent not only on the number of PMN, but also on the presence or absence of SEA, and also dependent on the prestimulation of the PMN (Fig. 10).

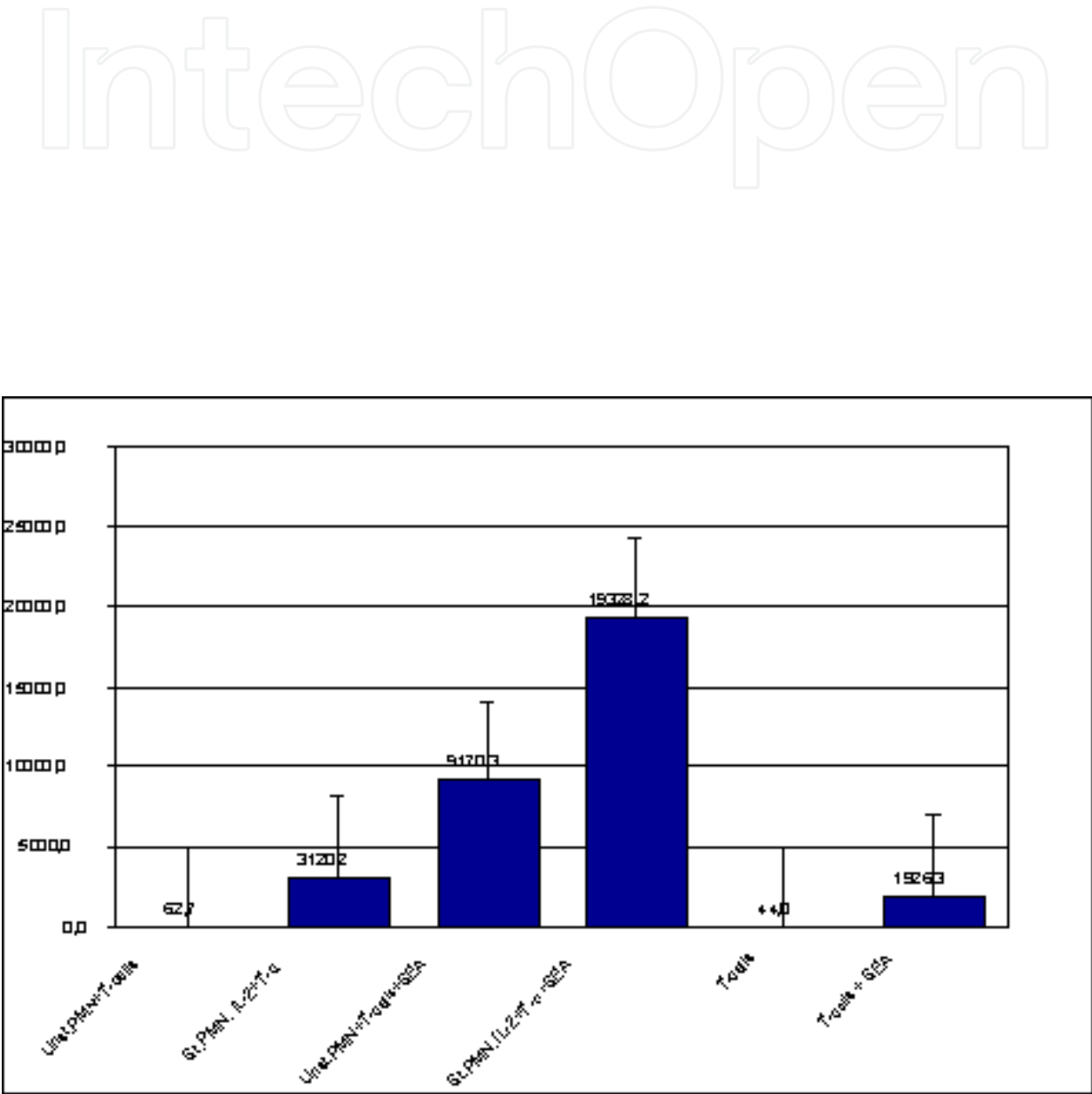


Fig. 10. Effects of bacterial Superantigens SEA on the proliferation of T-cells cocultured with  $1 \times 10^3$  PMN stimulated with IL-2.

Moreover, the T-cell proliferation could – at least to some extent – be inhibited, when the PMN had been cultivated with IL-2 in the presence of an antibody to the IL-2 receptor (Fig. 11).

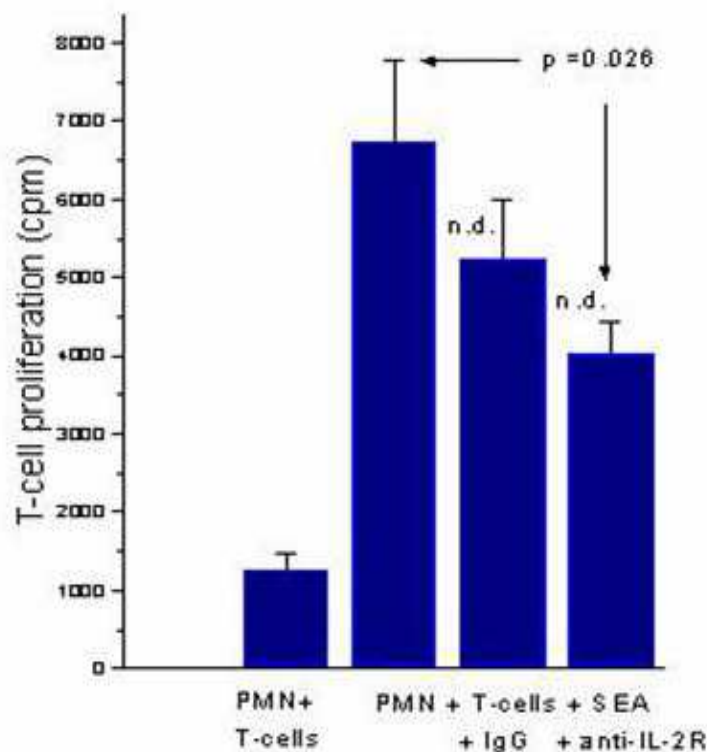


Fig. 11. Inhibition effect of anti-IL-2R on the proliferation of T-cells cocultured with  $1 \times 10^3$  PMN stimulated with IL-2 and bacterial superantigens SEA.

The above data indicated that a high level of IL-2 in our body increases immunity by its effect on PMN to act as antigen presenting cells by the expression of HLA class II and consequently increased T-cells proliferation (cell mediated immunity).

The differences between the groups in the sets of experiments related to the interaction of HLA class II positive PMN with peripheral T-cells were calculated by t-test using  $p < 0.05$  as limit.

#### 4. Discussion

PMN are important effectors cells in host defense and inflammation. They are considered short-lived cells undergoing spontaneous apoptosis *in vivo* as well as in culture (Savill, 1997). In recent years it has become increasingly evident that culturing PMN in the presence of cytokines extends their life span (Colotta et al., 1992; Klebanoff et al., 1992; Lee et al., 1993; Biffl et al., 1996).

Previous data by others suggested that PMN express a receptor for IL-2. The present study confirms these data and provides evidence that PMN express constitutively IL-2R. As shown for monocytes and NK cells only the IL-2R $\beta$  chain is expressed, but not IL-2R $\alpha$  (Espinoza-Delgado et al., 1990). This was in accordance with the present

immunofluorescence results, where we detected only IL-2R $\beta$  chain on resting PMN and confirmed my choosing of IL-2 as an activator of PMN.

Increasing evidence suggests that human PMN have an important role to play in regulating specific immune responses and in antigen presentation (Fanger et al., 1997; Radsak et al., 2000). In this study we have demonstrated that HLA Class II antigen was also detected, at the protein level by cytoflowmetry, and also at the gene level by RT-PCR. These observations therefore agree well with the findings of others who have detected these antigens on the surface of activated neutrophils (Matsumoto et al., 1987; Radsak et al., 2000). The detection of these molecules therefore provides strong support for the hypothesis that human PMN can actively synthesize immunoregulatory molecules (Newburger et al., 2000) and have the potential to act as APCs (Fanger et al., 1997; Radsak et al., 2000). The observation that PMN may also express HLA class II antigens when appropriately stimulated (Gosselin et al., 1993 and Fanger et al., 1997), highlighted the possibility that PMN might function as accessory cells for T-cell activation. HLA class II expression was found on PMN of patients with active Wegener's disease (Haensch, et al., 1999). The close correlation of HLA class II expression to disease activity prompted the present study with the objective of investigating whether or not HLA class II-positive PMN might activate or support activation of T lymphocytes.

Induction of the above membrane-bound molecules was higher when PMN were stimulated in whole blood. The explanation of this phenomena may be due to the loss of some PMN IL-2R and IL-15R and other activities during their isolation procedure.

The absence or low detection of MHC class II on human PMN stimulated with IL-2, IL-4 and IL-15 could be due to: 1) The relatively long time (44 hrs) required for class II induction, which is beyond the period of PMN survival in most culture systems. 2) IL-2 and IL-15 receptor numbers may vary with the individual. 3) Synthetic capacity of the PMN may vary. 4) The capacities of cytokine receptors to signal may not be the same. 5) It may also be possible that the PMN themselves produce factors that inhibit class II induction. 6) Synthetic capacity of the PMN may vary. 7) The capacities of cytokine receptors to signal may not be the same. 8) It may also be possible that the PMN themselves produce factors that inhibit class II induction (Gosselin et al., 1993).

When comparing the number of monocytes and PMN required inducing T-cell proliferation, it was observed that 10 times more PMN than monocytes were necessary to yield the same extent of T-cell proliferation. However, the cell preparation used in this work never contained more than 1% of contaminating cells and certainly not the 10% of monocytes that would be required to affect the results. The observation that ten times more PMN than monocytes were required to induce a similar extent of T-cell proliferation has to be considered together with the observation that only a proportion of PMN acquired HLA class II. Thus, when only fully equipped PMN are considered, the ability to process and to present antigen is similar to that of monocytes. Whether HLA class II-positive PMN participate in the immune defense or play a role in pathophysiological events, is a matter of speculation. The fact that only a minor proportion of PMN acquire HLA class II might lead to the conclusion that a possible accessory function of PMN would be rather weak. One has, however, to bear in mind that PMN are numerous in the peripheral blood, and that even a low percentage of PMN expressing HLA class II would exceed both circulating monocytes and dendritic cells in number.

Because of the notion that the presence of *Staphylococcus enterotoxin* coincides with relapses of Wagner's granulomatosis (Cohn-Terveart et al., 1999), we tested whether PMN was able to also present *Staphylococcus enterotoxin A* is a superantigen, so-called because it binds outside of the peptide-binding groove of the HLA class II and the antigen-specific domain of the T-cell receptor, and consequently activates a large portion of T-cells, preferentially those with a V beta 2 domain (Simpson et al., 1995). In accordance with previous studies (Fanger et al., 1997; Radsak et al., 2000), co-culture of PMN with T-cells and SE resulted in T-cell proliferation. Taken together, our data demonstrate that by synthesizing and expressing of HLA class II antigens, PMN acquire the capacity to present superantigens to T-cells.

## 5. Conclusion

In conclusion, PMN possession of IL-2R  $\beta$  and  $\gamma$  chains that have the ability to bind with IL-2, IL-4 and IL-15 prompted us to use IL-2, IL-4 and IL-15 as stimuli for PMN. They stimulated PMN to express HLA class II which is the main antigen presenting molecules. PMN expressed HLA class II present SEA to T-cells causing their proliferation. T-cells proliferations lead to antigen destruction, B-cells activation and consequently immunoglobulin production, and activation of phagocytic cells.

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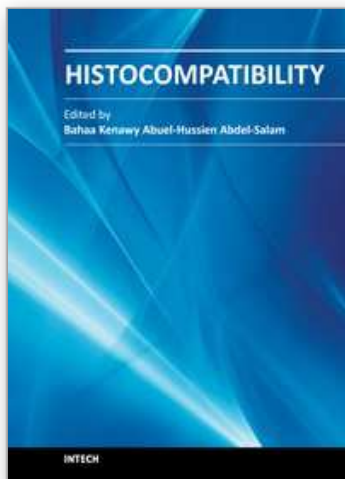
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