trends in hematology/oncology

haematologica 2002; 87:434-442
http://www.haematologica.ws/2002_04/434.htm

The interleukin-12 and interleukin-12 receptor system in normal and transformed human B-lymphocytes

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Background and Objectives. Interleukin-12 (IL-12) is a heterodimeric cytokine that induces interferon-γ (IFN-γ) production by natural killer and T-lymphocytes. IL-12 also activates human B-cells through the IL-12 receptor (IL-12R) complex. Here we review the expression and function of IL-12 and IL-12R in human B-cells and in their malignant counterparts.

Evidence and Information Sources. The information provided derives from results both published and unpublished obtained in the laboratories of the Authors, and from a comprehensive review of all the pertinent articles published so far in Medline.

State of Art. The two components of the IL-12R, i.e. the β 1 and β 2 chains, were found to be constitutively expressed in human naive, germinal center and memory tonsil B-cells; however, only naive B-cells were activated following interaction with IL-2. Here we show that the IL-12Rβ2 gene is not expressed in EBV-transformed normal B-lymphocytes and in Burkitt's lymphoma B-cell lines. IL-12 p35 and p40 transcripts were detected in all tonsil B-cell subsets, but only naive and memory B-cells produced IL-12. In this study, biosynthesis of IL-12 was investigated in tonsil B-cells, showing that the molecular weight of the mature heterodimeric IL-12 was similar to that of monocyte-derived IL-12, with minor differences possibly related to glycosylation. Finally, malignant B-cells from follicular and marginal zone lymphomas expressed IL-12 p35 and p40 transcripts, whereas only p35 mRNA was detected in mantle cell lymphoma.

Perspectives. Taken together, the studies herein reviewed indicate that human B-cells, at variance with their murine counterparts, can produce IL-12 following CD40 ligation. IL-12 p35 and p40 transcripts are found in B-cells from different lymphoproliferative disorders, but the evidence that the cytokine is produced at the protein level is poor. IL-12R is expressed in the main human B-cell subsets, but it is functional only in naive B-cells. Finally, the failure of transformed B-cell lines to express IL-12Rβ2 mRNA opens up new perspectives in the investigation of B-cell malignant transformation.

Key words: IL-12, IL-12 receptor, human B lymphocytes, B-cell lymphoproliferative disorders.

Interleukin-12 (IL-12) is a heterodimeric cytokine, originally identified as an IFN-γ inducing factor, that is composed of a disulfide-bound 35 kD sub-unit (p35) and a 40 kD sub-unit (p40),[1,2] and enhances innate and adaptive immunity.[3] IL-12 is primarily produced by professional antigen-presenting cells (APC), i.e. dendritic cells,[4] monocytes/macrophages[5] and, to a lesser extent, B-lymphocytes (see below); furthermore, other cell types such as neutrophils and keratinocytes can produce the cytokine.[6,7] The IL-12 p35 sub-unit is expressed ubiquitously, whereas expression of the p40 sub-unit is restricted to IL-12-producing cells.[3]

Both p35 and p40, beside being assembled in the IL-12 heterodimer, participate in the formation of other molecular complexes, e.g. p40-p40 homodimer,[8,9] p35-EBI3 heterodimer,[10] and p40-p19 heterodimer. The EBI3 gene, which is 27% homologous to the p40 gene, is expressed in EBV-infected B-cells, human placenta and stimulated monocytes, and its function is unknown (Table 1).[11] In humans, both of the above dimeric complexes are devoid of biological activity.[8-10] However, in mice, the p40-p40 homodimer functions as a competitive

IL-12 antagonist.[9,12] p40-p19 heterodimer, i.e. IL-23[13] (Table 1) is produced by activated human and murine DC, binds to the IL-12Rβ1, but not to the β2, chain and induces STAT4 activation. It is supposed that IL-23 uses its own unique receptor subunit in addition to IL-12Rβ1 to stimulate IFN-γ production and proliferation of PHA-activated T-cell blasts.[13] Finally, biologically inactive free p40 chains are secreted by IL-12-producing cells.[2,5,9]

IL-12 exerts immunoregulatory effects on T- and NK-cells by inducing IFN-γ production from both cell types, enhancing their proliferation and cytotoxicity, and driving T-helper cells to differentiate into the Th1 phenotype.[3] Furthermore, as will be discussed below, IL-12 can induce murine and human B-cells to produce IgM and IFN-γ.[3]

The IL-12 receptor (R) is composed of two subunits, named β1 and β2, that in humans are both required for high affinity IL-12 binding and signaling.[14-16] Studies in which the IL-12R β1 and β2 chain genes were expressed in COS-7 cells clearly demonstrated that IL-12Rβ1 is primarily responsible for binding, whereas IL-12R β2 is essential for signaling.[16]

IL-12Rβ1 is widely expressed in cells of hematopoietic origin,[17] whereas IL-12R β2 is detected selectively in cells responsive to IL-12, e.g. Th1 but not Th2 lymphocytes.[18,19] Both in humans and mice, IL-12 induces phosphorylation of Janus kinase 2, Tyk2 and STAT-4 in T- and NK-cells,[20-24] but an alternative signaling pathway, i.e. activation of NFκB components, has been demonstrated for murine DC[25] and human B-lymphocytes (see below).[26]

Studies carried out in gene knock-out mice have contributed significantly to the understanding of the functions of IL-12 and its receptor.[27-30] The phenotypes of IL-12 p35 or p40 deficient mice are similar to those observed in IL-12Rβ1 or β2 knock-out animals, i.e. all these animals display i) normal development and no apparent defects in lymphoid tissues; ii) impaired IFN-γ production and Th1 responses and, consequently, iii) increased susceptibility to infections with intracellular pathogens, such as *M. tuberculosis*, *M. avium* and *L. monocytogenes*. Comparative analyses of IL-12 p40 *vs* p35 and of IL-12Rβ1 vs β2 knock-out mice have shown that both IL-12 p35 and IL-12Rβ2 deficient animals show a less severe phenotype.[27-30] These observations suggest that IL-23 (i.e. the p19/p40 heterodimer, see above) can partially compensate for IL-12 deficiency by binding to its specific receptor, made up of IL-12Rβ1 and other component(s) so far unknown (see above). Finally, it is of note that human immune deficiency disorders due to mutations in the IL-12 p40 or IL-12Rβ1 genes have been identified.[31] In all cases reported, the mutations precluded protein expression; each patient suffered from severe infection with either non-tuberculous mycobacteria or vaccine-associated BCG, and most had severe Salmonella infections. However, in most instances, infections were effectively treated with antibiotics.

Table 1. Molecular complexes formed by IL-12, p35 and p40 with different partners.

Molecular form	*Receptor binding*	*Biological activity*	
		Mouse	*Man*
IL-12[1]	IL-12R	+	+
p40 monomer	unknown	–	–
p40 homodimer[2]	IL-12R	+	–
p35-EBI3	unknown	–	–
p40-p19[1]	IL-23R	+	+

[1]Agonist; [2]antagonist.

Table 2. IL-12 and IL-12R gene expression in normal tonsil B-cell subset and in transformed B-cells.

	Naive	*GC*	*Memory*	*EBV-infected LCLs*	*BL cell lines*
IL-12 p35	+	+	+	+	+
IL-12 p40	+	+	+	+	+
IL12Rβ1	+	+	+	+	+
IL12Rβ2	+	+	+	–	–

IL-12 gene expression and production in normal and transformed human B-lymphocytes

IL-12 was originally isolated as a product of the human EBV+ RPMI 8866 B-cell line.[1] Subsequently, IL-12 gene expression and production by human B-cells have been extensively investigated using a panel of normal EBV-infected lymphoblastoid cell lines (EBV-LCL) and of AIDS-associated Burkitt's lymphoma (BL) cell lines, either EBV+ or EBV–.[32] In this study, all B-cell lines were found to express the IL-12 p35 mRNA, whereas only EBV+ LCL and BL cells expressed the p40 transcript. Stimulation of EBV+ LCL and BL cell lines with PMA induced *de novo* secretion of IL-12 or increased its secretion, whereas EBV– BL cells remained unable to produce the cytokine under the same conditions. These results suggest that IL-12 production by transformed B-

cells is strictly dependent on EBV infection; it has been proposed that expression of latent membrane protein-1 (LMP-1) by EBV-infected B-cells plays a crucial role in triggering IL-12 production.[33]

The relationship between EBV infection and IL-12 production by human B-cells is emphasized by a recent study performed with biopsy specimens from patients with Sjögren's syndrome.[34] It was found that IL-12 was markedly expressed in the epithelial cells and in the infiltrating B-cells of salivary gland tissues from such patients, but not in those from normal individuals. A quite clear topographic correlation between EBV and IL-12 expression was observed.

Few studies have addressed IL-12 gene expression and production by fresh murine or human B-cells. In mice, it was shown that B-cells isolated from the lymph nodes of immunized animals did not produce IL-12.[35]

We studied the expression of p35, p40 and EBI3 mRNA in freshly isolated human tonsil B-cells and showed that these cells contain IL-12 p35 and p40,[36] but not EBI3, mRNA (Figure 1), suggesting that p35-EBI3 complexes cannot be formed in such cells. Tonsil B-lymphocytes are comprised of three major sub-populations which are distinguished according to immunophenotype, anatomic location and functional features.[37-41] These B-cell subsets, named germinal center (GC), naive and memory cells, are found in the germinal center, in the follicular mantle and in the subepithelial areas of the tonsil, respectively.[37-41] The CD38 surface marker allows GC ($CD38^+$) cells to be separated from non-GC ($CD38^-$) ones, i.e. naive and memory B-lymphocytes. The latter cells can be further fractioned according to the expression of surface IgD into naive (IgD^+) and memory (IgD^-) B cells.[37-41]

We investigated IL-12 p35 and p40, as well as EBI3, gene expression in naive, GC and memory B-lymphocytes, freshly purified from human tonsils. The three B-cell subsets constitutively expressed IL-12 p35 and p40 mRNA,[36] whereas, as expected, they lacked EBI3 mRNA (Figure 1).

IL-12 production by human B-lymphocytes was studied by Schultze *et al.*,[42] who demonstrated that a subset of tonsil B-cells (i.e. $CD38^-$, $IgD^\pm$ cells, that include naive and memory B-lymphocytes) is induced to express and secrete low amounts of IL-12 following CD40 ligation, but not following anti-immunoglobulin (Ig) monoclonal antibody (mAb) stimulation. In contrast, GC B-cells did not release the cytokine under any experimental condition.

The reason why GC B-cells behave differently from naive and memory B-cells as to IL-12 production, in spite of the constitutive expression of IL-12 p35 and p40 mRNA in the three B-cell subsets, is presently unknown and warrants further investigation.

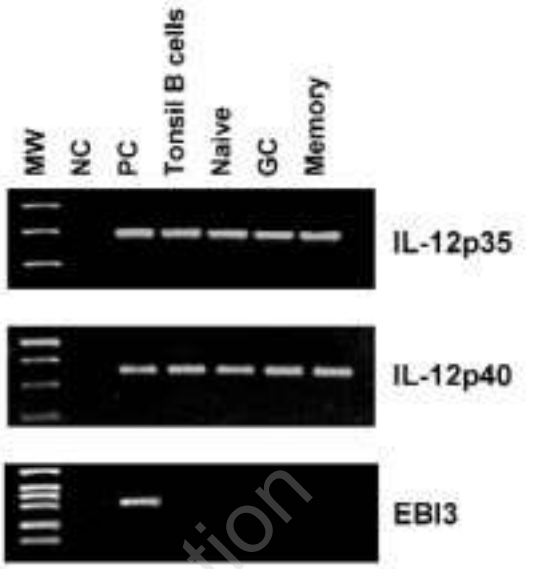


Figure 1. Expression of IL-12p35, p40 and EBI3 mRNA in freshly isolated tonsil B-cell subsets as assessed by RT-PCR. Left to right: molecular weight markers (MW); negative control (NC), represented by water in the place of cDNA; positive control (PC), represented by peripheral blood mononuclear cells stimulated with phytohemagglutinin for 6 h; total tonsil B-cells; naive (IgD^+), germinal center (GC, IgD^-, $CD38^+$), and memory (IgD^-, $CD38^-$) B-cells.

We have recently performed experiments to investigate IL-12 biosynthesis by spleen or tonsil human B-cells. To this end, purified B-lymphocytes were cultured with or without stimuli (IFN-γ and SAC) and labeled with ^{35}S-methionine; supernatants were subsequently run on an SDS-PAGE gel and immunoprecipitated with anti-IL-12 p40 and p70 mAbs; controls were peripheral blood monocytes stimulated and processed as above.

The results of one representative experiment are shown in Figure 2. In control monocytes (Figure 2, lane 1) the following bands were consistently detected: 36 kD and 39 kD, representing two discrete forms of the IL-12 p40 chain, and 80 kD, corresponding to the mature heterodimeric IL-12 molecule. Both 36 kD and 39 kD IL-12 p40 participate in the formation of the IL-12 heterodimer.[43]

Some differences emerged from the analysis of B-cell supernatants: i) the molecular weight of the predominant form of the IL-12 p40 chain was 35 kD

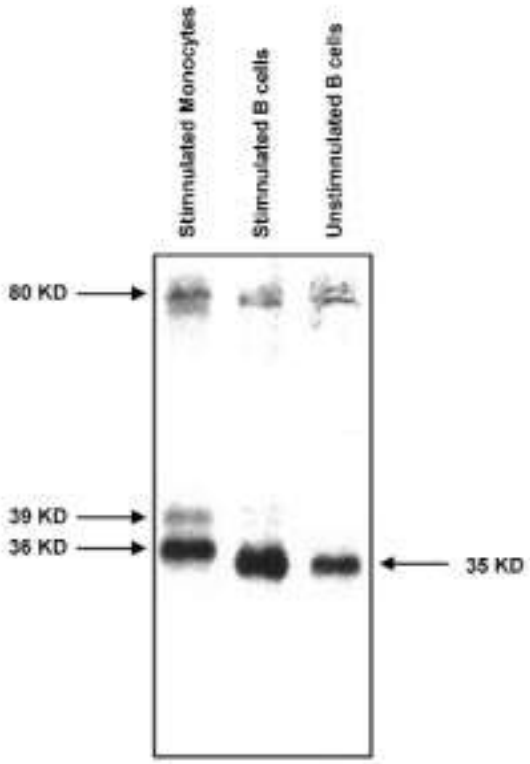


Figure 2. SDS-PAGE analysis of extracellular IL-12 heterodimer and free p40 chain. [35]S-methionine-labeled cell-free supernatants from IFN-γ primed monocytes stimulated with SAC (lane 1) or spleen-derived B-lymphocytes cultured without stimuli (lane 2) or with IFN-γ and SAC as above (lane 3) were immunoprecipitated with anti-IL-12 C11.79 mAb. See text for further explanation.

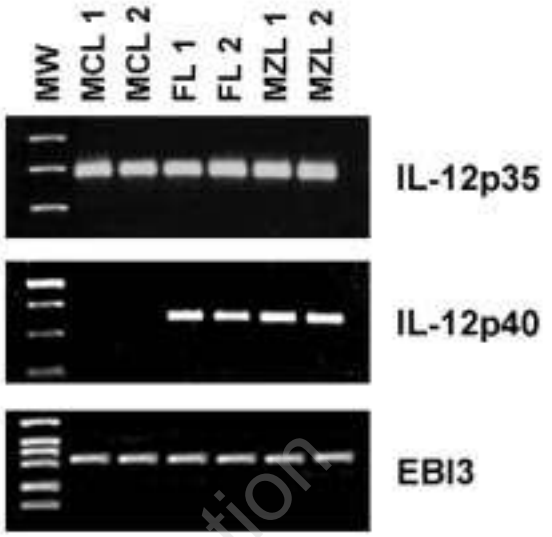


Figure 3. Expression of IL-12 p35, p40 and EBI3 mRNA in MCL, FL and MZL. From left to right: molecular weight markers (MW); MCL B-cells, case 1 (MCL 1) and 2 (MCL 2); FL B-cells, case 1 (FL 1) and 2 (FL 2); MZL B-cells, case 1 (MZL 1) and 2 (MZL2).

rather 36 kD (Figure 2, lanes 2 and 3), and ii) the 39 kD form of the IL-12 p40 chain was completely absent from the supernatants of resting B-cells and barely detectable in those of activated B-cells (Figure 2, lanes 2 and 3). Differential glycosylation processes may be involved in the different composition of the p35/36 band in B-cells vs monocytes. Notably, the intensity of the p35 band was clearly higher (from 1.88 to 2.1 fold) in the supernatants of activated B-cells than in those of resting B-cells (Figure 2).

Although the molecular weight of the mature heterodimeric IL-12 in B-cell supernatants was similar to that detected in monocyte supernatants, the possibility that the structure of the B-cell-derived IL-12 heterodimer is somewhat different from that of the monocyte-derived heterodimer cannot be excluded.

Taken together, the studies reviewed here demonstrate that human B-lymphocytes, at variance with murine B-cells, can produce IL-12,[35] especially following CD40 ligation.[42] On the other hand, B-cell-derived IL-12 is subjected to micro-environmental influences that can modulate its production; thus, for example, IFN-γ enhanced, whereas IL-10 blocked, CD40 ligand-triggered IL-12 production by naive and memory B-cells.[42]

In principle, IL-12 and IFN-γ produced by B-cells in the course of antigen presentation to T-helper lymphocytes might promote Th1 differentiation. However, the low amounts of cytokines available and the IL-12-induced production of its natural antagonist IL-10[3] support the general view that B-cell antigen presentation maintains the Th1 or Th2 phenotype of already differentiated $CD4^+$ cells or else induces Th2 differentiation by default.[42,44]

According to the *Revised European American Lymphoma* (R.E.A.L.) classification,[45] three types of human B-cell lymphoma originating from normal naive, germinal center (GC) and memory B-cells have been identified and designated as mantle cell (MCL), follicular (FL) and marginal zone (MZL) lymphomas, respectively. Malignant B-cells were purified from the invaded lymph nodes of MCL, FL and MZL patients and tested by reverse transcription

polymerase chain reaction (RT-PCR) for the constitutive expression of p35, p40[36] and EBI3 mRNA. As shown in Figure 3, all tumors expressed IL-12 p35 and EBI3 transcripts, whereas IL-12 p40 mRNA was consistently detected in FL and MZL, but not in MCL cells; *in vitro* stimulation of the latter cells with CD40 mAb and IL-4 failed to induce p40 mRNA expression.[36]

Since FL and BL are both believed to originate from germinal center B-cells,[45] the finding that freshly isolated FL B-cells expressed both IL-12 p35 and IL-12 p40 transcripts is at variance with the observation that EBV- BL cell lines expressed p35, but not p40, mRNA.[32] However, the two experimental models cannot be easily compared, in particular because all the BL cell lines tested in the latter study had been derived from HIV-infected patients.

Constitutive expression of the EBI3 gene in malignant B-cells from MCL, FL and MZL but not in their postulated normal counterparts, may reflect differences in the activation state of the former vs the latter cells or, alternatively, may represent a marker of transformed B-cells. Studies are now in progress to clarify this issue.

A previous study addressed IL-12 expression in human lymphomas using predominantly immunohistochemical techniques with mAbs to IL-12 p35, p40 and p70. In all samples tested, including a few cases of FL, MCL and MZL, no expression of the IL-12 heterodimer or of its components was detected in the neoplastic cells. However, in a large number of Hodgkin's disease cases, T-cells clustering around Reed-Sternberg cells were found to express IL-12.[46]

Collectively, the available studies on IL-12 production by malignant B-cells suggest that the IL-12 p40 gene is constitutively transcribed in some (FL, MZL), but not in other (MCL), lymphoma entities, whereas the p35 mRNA is always expressed. Apparently, however, there is no production of the IL-12 protein in malignant B-cells, suggesting that the IL-12 heterodimer is not synthesized by such cells, or is produced at levels below the threshold of detection of conventional assays.

Characterization of the IL-12R on normal and transformed human B-lymphocytes

Murine studies have demonstrated that IL-12 mediates various biological effects on B-cells, such as enhanced production of most antibody isotypes and changes in IgG subclass distribution during antigen-specific immune responses,[47] as well as *in vivo* depletion of $CD5^+$ peritoneal B1 cells.[48] It has been proposed that that the initial recruitment of new B-cell clones into the response to antigen is mediated by IFN-γ, whereas the subsequent enhancement of Ig secretion occurs in an IFN-γ independent fashion.[47] According to this model, IL-12 would stimulate T- and NK-cells to produce IFN-γ that, in turn, would promote the switch from IgG1 to IgG2a production and the temporary suppression of IgG1 production.[47] Thereafter, IL-12 would stimulate post-switched cells to produce large amounts of antibodies of different isotypes independently of IFN-γ.[47] Since B1 cells are known to inhibit competitively Ig secretion by conventional B-cells, the IL-12-induced depletion of B1 cells would represent an additional way to enhance antibody responses further.[47]

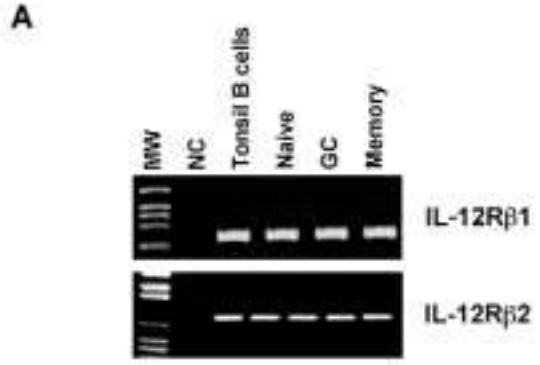
Numerous effects of IL-12 on human B-cells from normal individuals have been documented, namely i) induction of proliferation of pre-activated B-lymphocytes;[49,50] ii) induction of differentiation to Ig-secreting cells, especially in association with IL-2;[49,51] iii) induction of expression of the two IL-18R chains, i.e. interleukin-1 receptor related protein (IL-1RrP) and accessory protein like (AcPL);[26] iv) enhancement of CD25 expression;[52] v) induction of IFN-γ mRNA and protein;[49,52] vi) up-regulation of CD38 expression,[53] and vii) inhibition of IgE synthesis induced by IL-4.[54] With the exception of the last effect, which is probably indirect and mediated by T- or NK-cells, all of the other data were obtained using purified human B-lymphocytes.

Studies carried out in patients with hyper-IgE due to filariasis or helminthic infections have demonstrated that IL-12 suppresses IgE synthesis induced by polyclonal stimuli or parasite antigens through indirect effects.[55,56] Furthermore, in patients with systemic lupus erythematosus, IL-12 was found to inhibit the spontaneous *in vitro* production of IgM, IgG1, IgG2 and IgG3 through IFN-γ and IL-10-independent mechanisms.[57]

Although all of the above observations clearly pointed to the presence of functional IL-12R on murine and human B-cells, a detailed characterization of this receptor on such cells had not been carried out until recently. Furthermore, the initial observation that human IL-12 could bind to SAC-pre-activated peripheral blood B-lymphocytes[58] was not confirmed by other reports.

To fill this gap, we investigated the expression and function of IL-12R in human tonsil B-lymphocytes and in their major subsets.

RT-PCR analysis of freshly isolated tonsil B-cells demonstrated that they constitutively expressed both the IL-12R β1 and the β 2 chains.[26] Studies on β 1 and β 2 mRNA modulation were performed



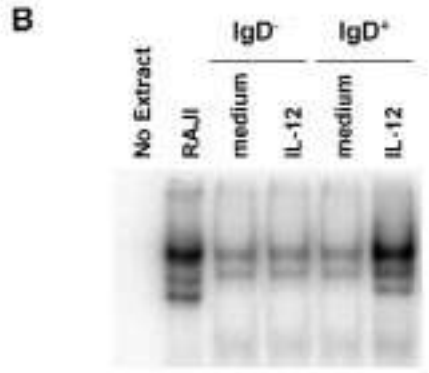


Figure 4. Expression of IL-12R β1 and β2 chain mRNA, and IL-12-induced NFκB activation in human tonsil B-lymphocytes. 4A. Expression of IL-12R β1 and β2 transcripts in freshly isolated B-cell subsets as assessed by RT-PCR. Left to right: MW, molecular weight; NC, negative control represented by water in the place of cDNA; total tonsil lymphocytes; naive (IgD⁺), germinal center (GC, IgD⁻, CD38⁺), and memory (IgD, CD38⁻) B-cells. 4B. Electrophoretic mobility shift assay (EMSA) performed on nuclear extracts from freshly isolated tonsil B-cell subsets stimulated with IL-12 for 30 min. The probe used has the sequence of the NFκB binding site of the Igλ light chain enhancer. From left to right: negative control (no extract); RAJI B-cell lines (positive control); IgD⁻ (GC and memory) B-cells incubated with medium (none) or IL-12; IgD⁺ (naive) B-cells incubated with medium (none) or IL-12.

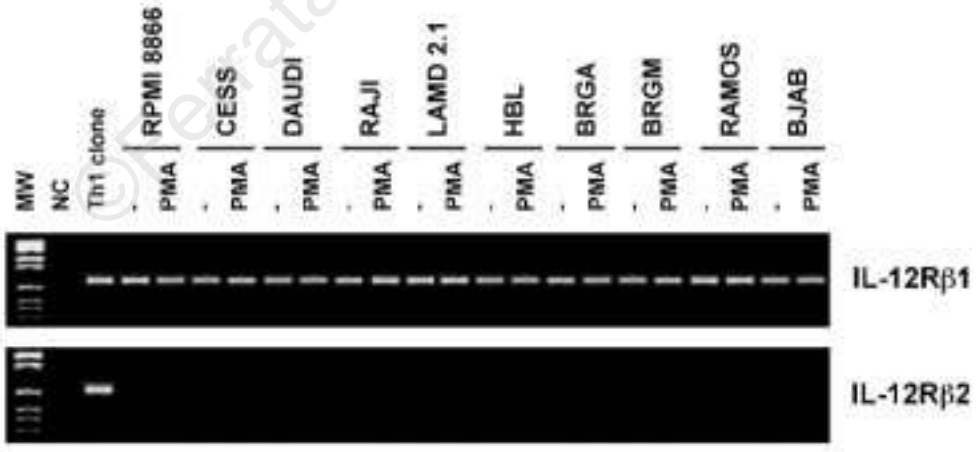


Figure 5. Expression of IL-12Rβ1 and β2 chain mRNA in transformed B-cell lines. From left to right: molecular weight marker (MW); an Ag-specific Th1 clone; RPMI 8866 EBV⁺ lymphoblastoid B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; CESS EBV⁺ lymphoblastoid B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; DAUDI EBV⁺ Burkitt lymphoma B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; RAJI EBV⁺ Burkitt lymphoma B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; LAMD2.1 EBV⁺ Burkitt's lymphoma B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; HBL EBV⁻ Burkitt's lymphoma B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; BRGA EBV⁻ Burkitt's lymphoma B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; BRGM EBV⁻ Burkitt's lymphoma B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; RAMOS EBV- Burkitt's lymphoma B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h; BJAB EBV- Burkitt's lymphoma B-cell line unstimulated (-) and stimulated with PMA (PMA) for 8 h.

using stimuli that mimic *in vitro* T-cell-dependent (CD40 mAb alone or in combination with IL-4) or –independent (anti Ig mAbs, SAC, IL-12) B-cell activation.[26] IL-12Rβ1 expression was not modulated by any of the stimuli, whereas IL-12Rβ2 mRNA was up-regulated up to 6 fold only following stimulation with SAC or IL-12 itself, as assessed by RNAse protection assay (RPA) analysis.[26]

When tonsil B-cell subsets were tested for IL-12Rβ1 and β2 mRNA expression, it was found that naive, GC and memory B-cells all contained both transcripts (Figure 4, panel A).[26]

In the same experiments, IL-12 was found to transduce signals after binding to IL-12R on the B-cell surface through a pathway different from STAT-4 (see below), that operates in T- and NK-cells. Notably, however, B-lymphocytes did phosphorylate and activate STAT family members, including STAT-4, upon stimulation with IFN-α. This finding indicates that the STAT pathway is functional in these cells.[26] In human tonsil B-cells, IL-12 activated p50 and c-Rel, two members of the NFκB complex which form the p50-p50 homodimer and p50-cRel heterodimer, both capable of binding to the promoter regions of different genes.[26] However, only naive B-cells showed NFκB activation following incubation with IL-12, whereas GC and memory B-cells did not[26] (Figure 4, panel B).

IL-12 induces IFN-γ production in murine and human B-lymphocytes,[26,49,50] especially in combination with IL-18.[26,59] Therefore, IFN-γ production was tested as a functional marker of B-cell subset activation by IL-12; in these experiments, we showed that only naive B-cells produced IFN-γ following incubation with IL-12, whereas GC and memory B-cells did not.[26] These results are consistent with those of the signal transduction experiments discussed above.

As mentioned, there is much evidence in support of a close relationship between EBV infection and IL-12 expression and it has been suggested that proteins encoded by the viral genome, e.g. LMP-1, are key regulators of IL-12 production in EBV-infected cells.[33] Therefore, the issue of whether or not EBV infection also affected IL-12Rβ1 and β2 mRNA expression in human B-cells was investigated using a panel of cell lines of different origins, either EBV-positive or negative.

B-cell LCLs (RPMI 8866 and CESS), as well as EBV$^+$ (DAUDI, RAJI and LAMD2.1) and EBV$^-$ (BRGA, BRGM, BJAB, HBL and RAMOS) BL cell lines, consistently expressed the IL-12R β1,[32] but not the β2 mRNA, and PMA stimulation did not induce the expression of the latter transcript, as assessed by RT-PCR (Figure 5). Taken together, these results indicate that EBV-infected LCLs and BL cell lines do not express IL-12R β2, irrespectively of whether they do or do not carry the EBV genome.

Concluding remarks

The data discussed in this review lead to the following conclusions:

1) human B-lymphocytes, at variance with murine B-cells, produce IL-12 following CD40 ligation; naive and memory, but not GC, tonsil B-cells synthesize the cytokine.[42] The functional relevance of B-cell-derived IL-12 in antigen presentation remains to be established;

2) IL-12R expression is detected in naive, GC and memory B-cells by RT-PCR, but the receptor is functional only in naive B-cells.[26] Notably, in this respect, murine CD5$^+$ B1 cells are a target of the *in vivo* activity of IL-12;[48] furthermore, they produce cytokines following *in vitro* incubation with IL-12;[60]

3) malignant B-lymphocytes often express IL-12 p35 and p40 transcripts, but do not produce the cytokine at detectable levels;[36,46] however, EBV-infected LCL or BL cell lines synthesize and release IL-12 following PMA stimulation, indicating a link between IL-12 production and EBV infection;[32]

4) EBV-transformed LCL and BL cells, either EBV$^+$ or EBV$^-$, do not express IL-12Rβ2 mRNA, even following PMA stimulation.

The latter findings, may be relevant in view of the use of recombinant IL-12 as an anti-tumor agent in B-cell lymphoproliferative disorders.[61]

Contributions and Acknowledgments

The authors wish to thank: Dr. Silvio Roncella and Dr. Manlio Ferrarini for providing a panel of Burkitt's lymphoma cell lines; Dr. Jason Marshall, Dr. Giorgia Gri, Dr. Paola Facchetti and Dr. Fabio Ghiotto for their contribution to some of the studies here reviewed.

Funding

This work was supported by grants from AIRC 2001, Ministero della Sanità, Progetti di Ricerca Finalizzata 1997 to V.P. and MURST 40% project to GC and FG, Italy.

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Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received October 17, 2001; accepted January 15, 2002.