The SH2 domain containing inositol 5-phosphatase SHIP2 associates to the immunoreceptor tyrosine-based inhibition motif of FcγRIIB in B cells under negative signaling

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Received 10 November 1999; accepted 21 December 1999

Abstract

FcγRIIB are single-chain low-affinity receptors for IgG that bear an immunoreceptor tyrosine-based inhibition motif (ITIM) in their intracytoplasmic domain and that negatively regulate immunoreceptor tyrosine-based activation motif (ITAM)-dependent cell activation. In B cells, coaggregation of the B cell receptor (BCR) and FcγRIIB leads to an inhibition of B cell activation. Inhibitory properties of FcγRIIB have been related to the recruitment of SHIP, an SH2 domain-containing inositol 5-phosphatase (referred to as SHIP1), via ITIM phosphorylated FcγRIIB. Here, we demonstrate that the second SH2 domain-containing inositol 5-phosphatase SHIP2 could also bind to the FcγRIIB ITIM. As a model, a FcγRIIB deficient B cell line (IIA1.6), transfected with a cDNA encoding either w.t. FcγRIIB1 or FcγRIIB1 whose ITIM tyrosine was mutated has been used. SHIP2 tyrosine phosphorylation and association to the adaptor protein Shc were only found in transfectants expressing w.t. FcγRIIB1. SHIP2 was also found to bind to a phosphopeptide corresponding to the ITIM sequence of FcγRIIB. There was no binding to the nonphosphorylated peptide. Finally, both SHIP2 and SHIP1 were coprecipitated with FcγRIIB1 upon coaggregation with BCR in IIA1.6 transfectants. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Phosphatidylinositol metabolism; Signal transduction; B cell; Fc receptor

1. Introduction

Fc receptors (FcR) recognize the Fc portion of antibodies (see [1] for a review). FcR capable of triggering cell activation possess immunoreceptor tyrosine-based activation motifs (ITAMs), which resemble those of the B cell receptor (BCR) and T cell receptor (TCR). FcγRIIB are a family of single-chain low-affinity IgG receptors that have no ITAMs and do not trigger cell activation. By various splice events, the gene encoding FcγRIIB generates three isoforms in mice, FcγRIIB1, FcγRIIB1’, FcγRIIB2, that differ only in a portion of their intracytoplasmic sequences. The intracytoplasmic domain of all FcγRIIB isoforms contain a motif termed immunoreceptor tyrosine-based inhibition motif (ITIM) that was shown to inhibit cell activation by all receptors with ITAMs [2], a phenomenon termed as ‘negative cooperation’. BCR aggregation by antigen gives rise to a sequence of intracellular events that leads to cell activation, and in response to signals delivered by coreceptors and cytokines, to cell proliferation and/or differentiation followed by antibody production. The coaggregation of BCR with FcγRIIB leads to a dominant negative signal that inhibits B cell activation [3–5]. Reconstitution experiments in IIA1.6 cells, a B cell line deficient for FcγRIIB expression, showed that murine FcγRIIB1, FcγRIIB1’ or FcγRIIB2 were equally inhibitory [6,7]. These findings support a model according

Abbreviations: BCR, B cell receptor; FcR, Fc receptors; Ins(1,3,4,5)P4, inositol 1,3,4,5-tetrakisphosphate; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based inhibition motif; PtdIns(3,4,5)P3, phosphatidylinositol 3,4,5 trisphosphate; RAM, rabbit anti-mouse IgG; SHIP, SH2-containing protein tyrosine phosphatases; SHIP2, SH2 domain containing inositol 5-phosphatase.

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to which FcγRIIB provides a powerful mechanism for B cells to discriminate between free antigen and antigen-antibody immune complex and thus to up or down-regulate immunoglobulin production.

The Src homology 2 (SH2) domain-containing inositol polyphosphate 5-phosphatase, SHIP (referred to as SHIP1) has been identified as a crucial negative regulator of BCR signaling [8–11]. SHIP1 is an inositol polyphosphate 5-phosphatase that uses as substrates inositol 1,3,4,5-tetrakisphosphate (Ins1,3,4,5P4) and phosphatidylinositol 3,4,5-trisphosphate (PtdIns3,4,5-P3). It is a 145 kDa tyrosine phosphorylated protein with several interesting features: an amino-terminal SH2 domain, a central catalytic domain, two phosphotyrosine-binding (PTB) consensus sequences, and three putative SH3-interacting proline-rich motifs at the carboxyl tail [12–16]. It has been demonstrated that coaggregation of BCR and FcγRIIB induces the phosphorylation of FcγRIIB on its ITIM, leading to the recruitment and tyrosine phosphorylation of SHIP1 [8,17]. SHIP1 can decrease the levels of PtdIns3,4,5P3 and thereby counteracts the recruitment and activation of the Bruton’s tyrosine kinase [10]. In the same model, SHIP1 has been reported to associate through its SH2 domain to Shc. This association can potentially prevent Grb2-Sos association to phosphorylated Shc and may thus inhibit Ras activation [18,19].

The cDNA of a second SH2 domain-containing inositol polyphosphate 5-phosphatase, referred to as SHIP2, has been cloned in humans [20] and, subsequently, in rats [21] and mice [22]. Its central catalytic region shows 64% amino acid identity with SHIP1 [23]. SHIP2 uses selectively as a substrate PtdIns(3,4,5)P3 [24,26]. Habib et al. [25] have reported that SHIP2 associates to Shc in growth factor- and insulin-stimulated human nonhematopoietic cell lines. Recent work by Wisniewski et al. [26] showed that stimulation of human hematopoietic progenitor cells with stem cell factor (SCF), interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) induced the rapid tyrosine phosphorylation of SHIP2 resulting in its association with Shc. We have observed that SHIP2 is expressed as a 160 kDa protein in both haemopoietic and non-haemopoietic cells and that, in murine B cells, SHIP2 is tyrosine phosphorylated and associates to Shc under negative signaling [27]. It was also observed that SHIP2 protein level, as determined by Western blotting, was increased after long term stimulation of human T lymphocytes, in contrast to SHIP1 which was not modulated under the same conditions [28]. To our knowledge, SHIP2, whose SH2 domain is only 54% identical to that of SHIP1, was not yet reported to bind to any transmembrane receptor. We tested here the hypothesis that SHIP2 binds to the ITIM of FcγRIIB by studying the behavior of SHIP2 during inhibitor of B cell activation, in a model of FcγRIIB-deficient murine B cells reconstituted with either FcγRIIB1' or an ITIM mutated form of FcγRIIB1. Our data establish that SHIP2 and SHIP1 are both recruited to the phosphorylated ITIM of FcγRIIB1'.

2. Materials and methods

2.1. Reagents

Rabbit polyclonal antibodies to Shc were obtained from Affinity (Manhead, Exeter, UK). Anti-rabbit IgG and anti-mouse IgG, horseradish peroxidase linked whole antibody, protein A Sepharose CL4B were obtained from Amersham Life Science (Roosendaal, The Netherlands). Antiphosphotyrosine monoclonal antibody 4G10 was purchased from Upstate Biotechnology (NY). Anti mouse SHIP1 and anti human SHIP2 antibodies were generated in rabbits as described previously [27]. Rabbit antibodies against recombinant EC domain of FcγRIIB were kind gifts from Dr Catherine Sautès (Institut Curie, Paris, France) and were used for Western blot analysis. The rat anti-mouse FcγRIIB 2.4G2 mAb was purified by affinity-chromatography on Protein G-Sepharose. 2.4G2 was coupled overnight to CNBr-activated Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden). Beads were saturated in 0.05 M ethanolamine and used to immunoprecipitate FcγRIIB1' (3 μl/1 × 10^7 cells).

2.2. Cell lines

The FcγRIIB-deficient mouse B lymphoma cell line IIA1.6. transfected with cDNA encoding FcγRIIB1' was previously described in [6]. ITIM mutated FcγRIIB1' was constructed in two steps. Sequences encoding the N-terminal part of the IC domain were first amplified using a 5' primer that hybridized with sequences encoding the nine N-terminal amino acids (primer N): AAG AAA AAG CAG GTA CCA CCA GCT CTC CCA, containing a KpnI site (underlined) and a 3' primer encoding the mutation tyrosine to glycine mutation (CTT GAG AAG TGA GCC GGT GAT CGT ATT). Sequences encoding the C-terminal part of the IC domain were amplified using a 5' primer encoding the mutation (AAT ACG ATC ACC GCC TCA CTT CTC AAG) and a 3' primer that hybridized with sequences encoding the 5 C-terminal amino acids and the first 16 nucleotides of the 3' untranslated sequences (primer C): GAG ACA CTA GAG CTC GGC TTT CTT GTC TTG C, containing a SacI site (underlined). The two overlapping PCR fragments were used as templates to amplify the whole mutated sequence using primers N and C. The resulting mutated fragment was cloned at KpnI and SacI sites into a NT vector containing se-
quences encoding the FcγRIIB EC and TM domains under the control of the SRα promoter as described [29] and transfected into IIA1.6. IIA1.6 cells were grown in RPMI 1640 medium supplemented with 1% L-glutamine, 5 mM sodium pyruvate, 50 µM 2-mercaptoethanol, 1% penicillin/streptomycin and 10% fetal bovine serum.

2.3. B cell activation and immunoprecipitation

IIA1.6 cells were stimulated with intact rabbit IgG anti-mouse IgG (RAM IgG) or F(ab')2 fragments of the same antibodies (RAM F(ab')2) (Organon Teknika). Immunoprecipitation of SHIP1, SHIP2 and Shc have been performed as reported previously [27]. Cells were washed in cold PBS and lysed in 10 mM Tris–HCl cold buffer, pH 7.5, supplemented with 150 mM KCl, 0.5% NP-40, 100 mM NaF, 2 mM mercaptopethanol, 1 mM Na3VO4, protease and phosphatase inhibitors (1 nM okadaic acid, 5 µM leupeptin, 0.1 mM pefabloc, 1 mM EDTA, 20 µg/ml calpain inhibitor I and II), for 30 min at 4°C under agitation. This was followed by centrifugation at 13 000 rpm/min for 10 min. The soluble fractions were used in immunoprecipitation. For FcγRIIB immunoprecipitation, the cells were lysed in 10 mM Tris–HCl cold buffer, pH 7.4, supplemented with 150 mM NaCl, 0.5% NP-40, 10 mM NaF, 10 mM sodium pyrophosphate, 2 mM Na3VO4, protease and phosphatase inhibitors (10 µg/ml aprotinin, 10 µg/ml pepstatin, 10 µg/ml leupeptin, 1 mM pefabloc, 1 mM EDTA), for 10 min at 4°C under agitation. Immunoprecipitates were boiled in Laemmli sample buffer and electrophoresed as previously described [27]. For SHIP1 and SHIP2 immunodetection, the blots were blocked with 5% milk powder in a buffer containing 10 mM Tris–HCl, pH 8, 150 mM NaCl and 0.05% NP-40. For phosphotyrosine and FcγRIIB revelation, milk was replaced by 5% bovine serum albumin. Western blots were incubated with the relevant antisera diluted 1/500 for SHIP1, 1/250 for SHIP2 or with antiphosphotyrosine (clone 4G10) diluted 1/1000, or with 3 µg/ml anti-FcγRIIB antibodies. Immunodetection were visualized using peroxidase-conjugated secondary antibodies and the enhanced chemoluminescence system (NEN Science Product, Boston, MA).

2.4. ITIM peptides and in vitro binding to proteins

Biotinylated peptides corresponding to the FcγRIIB ITIM (KTEAENTITYSLKLK) tyrosine phosphorylated or not were coupled to streptavidin-agarose beads. Beads were saturated with 1 mg/ml D-biotin, washed in buffer containing 10 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 1 mM Na3VO4, 5 mM NaF, 5 mM Na pyrophosphate, 0.4 mM EDTA, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 10 mg/ml pepstatin and 1 mM PMSF (lysis buffer) and incubated for 2 h in lysates from 1 × 107 cells. Beads were washed and boiled in sample buffer. Eluted material was fractionated by SDS-PAGE, transferred onto one Immobilon-P membrane (Millipore, Bedford, MA), saturated with 5% skimmed milk (Régilait, Saint-Martin-Belle-Roche, France) diluted in 10 mM Tris buffer pH 7.4 containing 0.5% Tween 20 (Merk, Schuchardt, Germany), and Western blotted with anti SHIP2 antibodies.

2.5. Flow cytometry

Cells were analysed by flow cytometry with a FACScan cytometer (Becton Dickinson, Mountain View, CA). The cells were incubated with FITC-coupled 2.4G2 (rat anti-mouse FcγRI/III, available through the ATCC, kindly provided by Dr Oberdan Leo, ULB). Cells were gated according to size and scatter in order to eliminate dead cells and debris from analysis.

3. Results

3.1. Specificity of antibodies towards SHIP2 in IIA1.6 cells

Cell lysates were prepared from IIA1.6 B cells and immunoprecipitated with anti SHIP1 or SHIP2 antibodies. Antibodies to SHIP1 and SHIP2 immunoprecipitated 130–145 kDa and 160 kDa protein bands, respectively, in IIA1.6 cells when probed with our antibodies against SHIP1 and SHIP2 (Fig. 1A). The SHIP2 peptide (1 µg/ml) used as an immunogen specifically prevented SHIP2 but not SHIP1 immunoprecipitation (Fig. 1A). The 160 kDa SHIP2 protein band was not detected after immunoprecipitation with our SHIP1 antibodies. The data therefore indicate that under the experimental conditions tested, our SHIP1 and SHIP2 antibodies do not crossreact in IIA1.6 cells as shown before in A20 B cells [27].

3.2. Both tyrosine phosphorylation of SHIP2 and its association with Shc in response to BCR-FcγRIIB coligation depends on the ITIM of FcγRIIB

In A20 B cells, SHIP2 was tyrosine phosphorylated and associated to Shc in response to BCR and FcγRIIB coaggregation [27]. The same was observed with SHIP1 and the kinetics of the two biochemical events were comparable. This could be due to (i) the interaction of SHIP1 and SHIP2 to the same protein(s), (ii) the interaction between SHIP1 and SHIP2 or (iii) the binding of SHIP2 to the ITIM of FcγRIIB. The possible direct binding of SHIP2 to the ITIM of FcγRIIB has been tested in this study.
The tyrosine phosphorylation of SHIP2 and its possible interaction with Shc in IIA1.6 cells, has been analysed in IIA1.6 cells transfected with cDNA encoding FcγRIIB1' or an ITIM-mutated form of FcγRIIB1' (see Fig. 1B for a flow cytometry analysis of FcγRIIB expression by these cell lines). This model offers the

![Immunoprecipitation of SHIP1 and SHIP2 present in IIA1.6 B cells.](image)

**A.** Immunoprecipitation of SHIP1 and SHIP2 present in IIA1.6 B cells. (A) IIA1.6 B cells (i.e. 10⁸ cells) were lysed and immunoprecipitated with anti SHIP1 or SHIP2 C-terminal antibodies: I.P. SHIP1; I.P. SHIP2, in the presence or absence of 1 μg of SHIP2 C-terminal peptide used as an immunogen. Samples were resolved on SDS-gels and probed with anti SHIP2 or anti SHIP1 antibodies, as indicated. The data are representative of two separate experiments. The positions of molecular mass markers are indicated (in kDa) at the left. SHIP1 and SHIP2 molecular masses (130–145 and 150–160 kDa, respectively) are indicated by arrows. (B) The expression of FcγRIIB1' in each populations of IIA1.6 cells was analyzed by one-color immunofluorescence following staining with FITC-labeled 2.4G2 antibody.

![Flow cytometry analysis](image)
Fig. 2. Tyrosine phosphorylation of SHIP1 and SHIP2 proteins in response to coaggregation of BCR and FcγRIIB was mediated by the ITIM of FcγRIIB. (A) FcγRIIB1' transfected IIA1.6 cells (i.e. $2 \times 10^8$ cells) were incubated for 2 min with media, intact RAM or F(ab')2 fragments of RAM. The cells were lysed and immunoprecipitated with anti SHIP2 antibodies (I.P.). Half of the immunoprecipitate was probed with anti phosphotyrosine (anti P-tyrosine). The other half was probed with anti SHIP2 antibodies as control. The data are representative of two separate experiments. (B) Wild type FcγRIIB1' transfected, ITIM mutated FcγRIIB1' transfected or control IIA1.6 cells (i.e. $2 \times 10^8$ cells) were incubated for 2 min with media or intact RAM, as indicated. The cells were lysed and immunoprecipitated (I.P.) with anti SHIP1 or SHIP2 antibodies. From half of the immunoprecipitate, blots were probed with anti phosphotyrosine. From the other half, blots were probed with anti SHIP1 or anti SHIP2 antibodies as control. The data are representative of four separate experiments.
possibility to analyse in the same cells, the importance
of the FcγRIIB inhibitory motif ITIM on the behavior
of both SHIP1 and SHIP2.

The effects of BCR aggregation by RAM F(ab')2 or
cogagregation of BCR with FcγRIIB1' by intact RAM
IgG, on tyrosine phosphorylation of SHIP2 have been
compared (Fig. 2A). As previously observed in A20
cells [27], in IIA1.6 cells transfected with FcγRIIB1',
phosphorylation of SHIP2 was observed 2 min after
cogagregation of BCR with FcγRIIB1', but not after
aggregation of BCR.

We then compared the effect of cogagregation of
BCR and FcγRIIB on tyrosine phosphorylation of
SHIP1 and SHIP2 in wild type and transfected IIA1.6
cells. A major tyrosine-phosphorylated band of about
160 kDa was immunoprecipitated from IIA1.6 cells
transfected with FcγRIIB1' with anti SHIP2 antibodies
and detected with an antiphosphotyrosine antibody 2
min after stimulation with intact RAM IgG (Fig. 2B).

SHIP1 phosphorylation tested in parallel presented similar results with a major phosphorylated
band of 145 kDa in cells transfected with FcγRIIB1'
and only when cells were stimulated by intact RAM
IgG, in agreement with previously published data (see
e.g. [3,30]). The same amounts of SHIP2 (or SHIP1)
were detected after immunoprecipitation, as shown
when the blots were probed with SHIP2 (or SHIP1)
antibodies, respectively (Fig. 2B).

Based on our previous work [27], we have tested the
association of SHIP1 and SHIP2 with Shc at 2 min of
stimulation in the IIA1.6 model. SHIP1 and SHIP2
were not detected in Shc immunoprecipitates from un-
stimulated cells (Fig. 3). When cells were stimulated by
intact RAM IgG, SHIP1 and SHIP2 were detected in
anti-Shc immunoprecipitates of FcγRIIB1' transfected
IIA1.6 cells. This coprecipitation was markedly reduced
in the absence of FcγRIIB1' or in presence of the ITIM
mutated FcγRIIB1' (Fig. 3).

\[
\begin{array}{ccc}
\text{FcγRIIB1':} & \text{w.t.} & \text{ITIM (-)} \\
\text{RAM IgG:} & - & - & - & + & + & +
\end{array}
\]

3.3. SHIP2 associates to FcγRIIB upon cogagregation
with BCR in FcγRIIB1' transfected IIA1.6 cells

In order to determine if SHIP2 could bind to
FcγRIIB, FcγRIIB was immunoprecipitated in
FcγRIIB1' transfected IIA1.6 cells stimulated by intact
RAM IgG. Antibody against SHIP2 detected a 160
kDa band in cell lysate immunoprecipitated with an
antibody to FcγRIIB (Fig. 4A). No signal could be
observed in unstimulated cells and similar data were
obtained with anti SHIP1 antibodies (with a 145 kDa
band as indicated by an arrow). When the same blot was probed with anti FcγRIIB antibodies, it showed the presence of the FcR in the two conditions tested. In addition, it was observed that a phosphopeptide corre-
sponding to the ITIM sequence of FcγRIIB could interact with SHIP2 present in a lysate of FcγRIIB1′ transfectected IIA1.6 cells (Fig. 4B). This was not ob-
served with the corresponding non phosphorylated pep-

Fig. 4. Association of SHIP1 and SHIP2 proteins to FcγRIIB in the ITIM sequence in response to coligation of BCR and FcγRIIB. (A) FcγRIIB1′ transfectected IIA1.6 cells (i.e. 2 x 10^8 cells) were incubated, for 2 min with media or intact RAM, as indicated. Immunoprecipitation (I.P.) was performed with 2.4G2 coupled to Sepharose or, with anti SHIP1 or anti SHIP-2 antibodies (for control expression of the two phosphatases on the same gel). The samples were analysed by Western blotting probed with anti SHIP1, anti SHIP2 antibodies or anti FcγRIIB, as indicated. The data are representative of three separate experiments. B. IIA1.6 cells (i.e. 10^7 cells) were lysed as described in Section 2. Precipitation of the adsorbed proteins was performed using agarose beads coupled to the ITIM peptide (non phospho) or phosphopeptide (phospho). The samples were analysed by Western blotting probed with anti SHIP2 antibodies.
tide. Taken together, these data indicate that, like SHIP1, SHIP2 can bind to the ITIM of FcγRIIB.

4. Discussion

The phenomenon of negative cooperation was first recognized between FcγRIIB and BCR. It has been recently demonstrated that FcγRIIB inhibit lymphoid and myeloid cell activation via TCR [2], via FcR with ITAM [31] and via c-kit [32]. The mechanism of FcγRIIB inhibition appears to be complex. The phospho-ITIM of murine FcγRIIB recruits SHPI1 [17] and possibly small amounts of SH2-containing protein tyrosine phosphatases SHP-1 and of SHP-2 [33], suggesting that several pathways may be triggered by FcγRIIB in the same cells. Recent work indicates that FC-R1IP-mediated inhibition in B cells can occur in the absence of SHP-1 but requires SHPI1 [34]. In the mouse, the role of SHP-2 in BCR-mediated cell activation and in FcγRIIB-mediated inhibitory signaling is still unclear [35]. A series of data support a possible function of SHP2 in B cells: (i) PtdIns(3,4,5)P3 dependent BCR activation of Akt kinase is inhibited by coaggregation with FcγRIIB. A slight inhibition of Akt was conserved in SHIP1-deficient cells [11] suggesting the participation of other 5-phosphatase, possibly SHIP2, in FcγRIIB negative signaling. (ii) We [27] and others [26] have described a similar distribution and behavior of both SHIP1 and SHIP2 in lymphoid cells. In particular, both SHIP1 and SHIP2 show PtdIns(3,4,5)P3 5-phosphatase activity. (iii) We demonstrate here that, like SHIP1, SHIP2 binds to the phospho-ITIM of FcγRIIB under negative signaling. We also show that both tyrosine phosphorylation of SHIP2 and its association with Shc are ITIM dependent. This similar behavior of SHIP1 and SHIP2 suggests a possible role for SHIP2 in the negative regulation of immunocompetent cells mediated by FcγRIIB and, more generally, in the negative feedback control of the immune response. However, SHIP1 and SHIP2 have a different hierarchy of binding to SH3 containing proteins and therefore may modulate different signaling pathways and/or localize to different cellular compartments [26].

In conclusion, we show that, in B cells, SHIP2 presents a similar behavior as compared to SHIP1 (i.e. ITIM dependent tyrosine phosphorylation, Shc association and FcγRIIB association) in the FcγRIIB inhibition pathway. At present, we could propose several hypotheses about the functional role of SHIP2 in FcγRIIB-dependent negative regulation of B cell activation. SHIP2 could compete with SHIP1 for the binding of FcγRIIB phospho-ITIM and for the binding of Shc and thus act as an inhibitor of SHIP1-dependent pathways. On the contrary, based on the similar inositol phosphatase specificity and activities of both inositol 5-phosphatases, SHIP2 could synergize with SHIP1 in negative signalling. In addition, SHIP2 could also functionally replace SHIP1 in cells expressing FcγRIIB but only low or undetectable levels of SHIP1.

Acknowledgements

We would like to thank Dr. Catherine Bruyns for many discussions, Dr. Catherine Sautès (Institut Curie, Paris, France) for FcγRIIB specific antibodies. This work is supported by grants of the Fonds de la Recherche Scientifique Médicale, Action de Recherche Concertée of the Communauté Française de Belgique, by the Belgian Program on Interuniversity Poles of Attraction initiated by the Belgian State, Prime Minister’s office. E.M. is supported by a grant from E.U. Biomed 2 programme BMH4-CT-97 2609. X.P. is supported by a grant Télémie (FNRS). P.B. is a recipient of a fellowship from the Association pour la Recherche contre le Cancer.

References