Peer reviewed article

Early lymphocyte development in bone marrow and thymus

Antonius G. Rolink, Steffen Massa, Gina Balciunaite, Rod Ceredig

Department of Clinical and Biological Sciences, University of Basel

Summary

Haematopoietic stem cells (HSCs), a very rare cell type in the bone marrow, are responsible for the life-long production of all cells of the blood including T and B cells. Until recently, it was thought that the differentiation of HSCs into the various haematopoietic cells was rather hierarchical in that differentiation along a given lineage was associated with a progressive loss of potential to give rise to other blood cell lineages. The recent development of very sensitive and quantitative in vitro assays, together with the identification of new progenitor subpopulations, has challenged this idea. Thus, lymphocyte progenitors can be shown to keep their developmental potential to give rise to myeloid, dendritic and NK cells until just prior to their final commitment stage. Here we review these new findings and concepts.

Key words: stem cells; bone marrow; thymus; T cell progenitors; B cell progenitors

Introduction

HSCs are responsible for the lifelong production of the various blood cells. Thanks to work from several laboratories the phenotype, as well as the relative numbers, of HSCs in the mouse and human BM are well established [1–3]. Thus, in mice HSCs are characterised by the cell-surface expression of Thy 1, c-kit and Sca 1 and the absence of B220, Gr-1, Ter119 and CD3, 4, 5, 8 and 11b. *In man, although the frequency of HSC is apparently lower, their absolute number would seem to be similar to that of mice [3]. Human HSCs express Thy, c-kit and variably CD34 [4–6] and do not express CD10, 14, 15, 16, 19 and 20 [2, 8]. In mice, HSCs comprise about 0.01% of all nucleated bone marrow cells and transplantation of single HSC has unambiguously demonstrated that they are able to produce all cells of the blood long-term [8, 9]. Clearly, these experiments are impossible in man.*

Differentiation of HSCs into the various haematopoietic lineages is usually pictured in a hierarchical fashion, in that cells develop first into progenitors and then into precursors, with decreasing pluripotency and increasing commitment to a single differentiation pathway. This idea was supported by the identification of a common lymphoid progenitor (CLP) [10] and a common myeloid progenitor (CMP) [11]. CLP could differentiate into T, B, and natural killer (NK) cells but not into myeloid cells. CMP, on the other hand, could give rise to various cells of the myeloid lineage but not to the lymphoid lineage. However, recent findings have challenged this hierarchical model of blood cell development. Probably the best examples showing that haematopoietic development has much more plasticity are studies performed in mice deficient for the transcription factor Pax5. Thus the *Pax5*$^{-/-}$ mouse has an absolute block in B cell development at the pro B cell stage. The pro B cells present in the bone marrow of *Pax5*$^{-/-}$ mice express RAG-1 and RAG-2 and transcripts for the B cell specific genes *λ5*, *VpreB*, and *Igα* and *Igβ* and have their Ig heavy chain loci D-J rearranged. *Rearrangement of D-J IgH and TCRβ genes are not hallmarks of B or T cell commitment respectively. Some time ago, IgH rearrangements were found in developing thymocytes [12], and we have recently shown TCRβ rearrangements in macrophages derived from progenitor thymocytes [13].* Moreover, like wild type pro-B cells, *Pax5*$^{-/-}$ pro-B cells can be grown in vitro on stromal cells in the presence of IL-7 for long periods of time. However, and in marked contrast to wild type cells, *Pax5*$^{-/-}$ progenitor B cells can develop into myeloid, NK, and T cells both in vitro and in vivo [14–16]. Recently, Busslinger and colleagues showed that even wild type precursor B cells regain this multilineage developmental potential upon conditional inactivation of the *Pax5*$^{-/-}$ gene [17]. These findings indicate that haematopoietic differentiation has much more plasticity than previously anticipated, i.e. progenitor cells on the way to differentiating into a given lineage remain able to give rise to other cell

This work was supported by the NRP46 grant of the Swiss National Science Foundation (NRP 46 Stem Cell Transplants 4046-101112). A. G. Rolink is holder of the Chair in Immunology endowed by Hoffmann-La Roche Ltd, Basel.

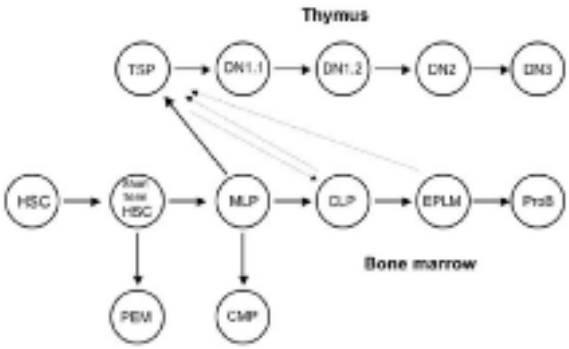


Figure 1

Current working model of haematopoietic development.

types until a rather advanced stage of differentiation [18]. *That a terminally differentiated T or B cell can be reprogrammed to produce an adult cloned animal has indeed been demonstrated [19, 20]. Whether such extreme de-differentiation could occur under physiological conditions is unclear.*

The establishment of highly efficient and quantitative in vitro assays to test the B, T and myeloid cell developmental potential of early mouse progenitor cells has also enormously improved our understanding of hematopoiesis. Thus a whole series of stromal cells are currently available that in the presence of IL-7 promote B, and in the presence of M-CSF promote myeloid cell development [13, 21]. Moreover, Schmitt and Zuniga-Pflucker recently generated an OP9 stromal cell line expressing the Notch ligand delta-like-1 (OP9-DL1) [22, 23]. Unlike wild type OP9 stromal cells, which very efficiently reveal a progenitor cell's B cell developmental potential, the OP9-DL1 stroma also allows a cell's T cell progenitor potential to be revealed.

In figure 1 our current working model of haematopoietic development is depicted. Thus HSCs with long-term self-renewing capacity give rise to ones with only limited self-renewing potential. Recent work by Adolfsson et al. [24] and by the Katzura Laboratory [25] has indicated that the short-term self-renewing HSC gives rise to a progenitor with erythroid and myeloid (PEM) potential. The next cell in our scheme is a progenitor with a developmental potential restricted to the myeloid and lymphoid lineages (MLP). These cells are the direct precursors of the common myeloid progenitor (CMP), a cell type with a developmental potential restricted to the myeloid lineages. MLPs will moreover give rise the common lymphocyte progenitors (CLP), a cell which in our opinion mainly gives rise to B cells, and some of the MLPs will migrate to the thymus (TSP: thymic seeding progenitor) and will there undergo the T cell differentiation programme. Early stages of T and B cell development will be described in more detail below. The solid arrows in the model indicate the main developmental pathway. The broken arrows indicate the lymphocyte precursors in the bone marrow that still possess T cell developmental potential and thymocyte precursors that possess B cell developmental potential.

Early stages of lymphoid development in mouse bone marrow

As mentioned above, pro-B cells found in the bone marrow of *Pax5*$^{-/-}$ mice show a remarkable degree of developmental plasticity. However, until recently it was unclear whether cells of this type were unique to the *Pax5*$^{-/-}$ mouse or whether they were present in wild type mice. Our laboratory has analysed in great detail whether a cell equivalent to the *Pax5*$^{-/-}$ pro-B cell is present in wild type bone marrow. *Pax5*$^{-/-}$ pro-B cells express B220 and c-kit (CD117) and are negative for CD19 and NK1.1. In fact, expression of CD19 is under direct transcriptional control of *Pax5* and can therefore be used as a surrogate marker for *Pax5* expression [26]. In the bone marrow of wild type mice about 0.2% of the nucleated cells have a phenotype of this kind. Moreover, like *Pax5*$^{-/-}$ pro B cells, these cells express the IL-7Rα (CD127), Flt3 (CD135) and CD93. In vitro analysis using the established culture systems to test B, T and myeloid developmental potential revealed that these cells from wild type bone marrow could in very high frequencies differentiate into all three lineages [27]. These findings suggested that the multi-lineage developmental potential of these cells was similar to that of the *Pax5*$^{-/-}$ pro B cells. In fact, their plasticity was even greater in that they could still switch on the *Pax5* gene and very efficiently give rise to B lineage cells. Given the fact that these cells from wild type mice can differentiate into B, T and myeloid cells, we have called them early progenitors with lymphoid and myeloid developmental potential, or EPLM.

EPLM in wild type bone marrow comprise about 0.2% of all nucleated cells and expressed CD135, the receptor for Flt3 ligand (Flt3L) [27]. Daily treatment of mice with 5–10 μg FLT3L for 7–10 days increased EPLM number fiftyfold [28]. In vitro analysis also revealed that these cells from Flt3L-treated mice possess very efficient myeloid and T cell developmental potential. However, their ability to generate B cells was dramatically reduced, thus accounting for the decreased precursor B cell compartment in the bone marrow of Flt3L-treated mice. Thus, the number of EPLMs in the bone marrow appears to be controlled by Flt3L and high levels of this cytokine seem to impair their B developmental potential.

EPLM in vitro can generate B, T and myeloid cells. In vivo transplantation studies, however, showed that low numbers ($2–5 \times 10^3$) of EPLM could only give rise to B cells while higher numbers (2×10^4) were able to generate T and B cells

Table 1

Cell-surface markers expressed by early lymphocyte progenitors in bone marrow.

	c-kit CD117	FLT3 (CD135)	CD93	B220	IL-7Ra (CD127)	CD44	CD25
CLP	+	+	++	–	+	+	–
EPLM	+	+	++	+	+	+	–
ProB	+	–	+++	++	+	+	–

Table 2

Developmental potential of early lymphocyte progenitors in the bone marrow.

	B	T	Myeloid	NK
CLP	+++	++	++	+
EPLM	+++	++	++	+
ProB	+++	–	–	–

[27]. No myeloid development of EPLM in vivo has thus far been observed. In the light of these findings we strongly favour the idea that under physiological conditions the developmental fate of EPLM is mainly to become B cells. Since in our hands CLPs have a very similar developmental potential to EPLMs, these cells should probably also be regarded as very early B cell progenitors [27]. Table 1 summarises the surface marker used to distinguish the early lymphocyte progenitor in the bone marrow.

Table 2 summarises the developmental potential of these early lymphocyte progenitors in the bone marrow.

Early stages of T cell development in the thymus

Early stages of T cell development in the thymus are characterised by the differential expression of the markers CD44 and CD25 and the absence of CD4, 8 and 3. Because they do not express either CD4 or CD8, these cells are usually called double negative (DN) thymocytes. DN thymocytes can be subdivided into four populations called DN1–4. DN1s are $CD44^+CD25^-$, DN2s are $CD44^+CD25^+$, DN3s are $CD44^-CD25^+$ and DN4s are $CD44^-CD25^-$. However, B, NK, myeloid and dendritic cells are also present in the thymus and most of them would have DN1-like phenotype. Hence the inclusion of c-kit (CD117) as a marker to define bona fide DN1 cells is absolutely crucial. DN1 and DN2 cells express high levels of c-kit, while DN3 cells express intermediate levels and DN4 cells are negative [29]. *The thymus produces T cells expressing T cell receptor heterodimers of TCRαβ or TCRγδ chains. Although cells expressing TCRγδ chains arise earlier in development and have particular cell tropisms, the exact cellular origin of TCRγδ cells within the progenitor compartment is still controversial [30]. However, the recent advent of transgenic mice containing a reporter gene within the TCRδ locus should help clarify this long-standing issue [31].*

Because the thymus does not harbour HSCs, a constant influx of progenitor cells from the bone marrow into the thymus is required to maintain T cell production. Over the last few years a whole series of papers have been published dealing with the phenotype and developmental potential of the bone marrow progenitor cell that enters the thymus [25, 32–39]. Studies by Radtke and colleagues showed unquestionably that signalling via the Notch1 receptor was an absolutely crucial event in early T cell commitment and development [36]. Thus, these authors showed that in Notch1 deficient mice the earliest thymocyte subpopulation was absent and that the thymi contained B cell progenitors and precursors. On the basis of these findings it was concluded that the thymus is seeded by a bone marrow precursor that still possesses B cell developmental potential and would lose this potential upon Notch signalling. However, several groups, including our own, have sought in vain in the adult thymus for progenitor cells with B developmental potential [13, 34, 35]. It should also be noted, however, that other groups have found low frequencies of cells able to give rise to B cells in the thymus [32, 33, 37, 39]. Recently Sambandam and colleagues [37] showed that a small fraction of the DN1 population also expresses CD135 and that it is this (DN1.1) subpopulation that contains B lineage potential. Moreover, these cells also appear to express the chemokine receptor CCR9, which may be the receptor that guides progenitor cell homing to the thymus [34].

We have recently been able to confirm this finding. In our hands CD135 is expressed by about 20% of DN1 cells and it has been proposed that CD135-positive DN1 cells are the precursors of the CD135-negative subpopulation. In our schemes of thymocyte development (see figure) we therefore subdivide DN1 cells into $CD135^+$ DN1.1 and $CD135^-$ DN1.2 cells. Limiting dilution analysis revealed that 1 in 1000 $CD135^+$ DN1.1 cells from adult mice were able to generate B lineage cells and these cells are TSP. In numerical terms this means that the adult thymus only harbours about 5 cells with B cell developmental potential. In marked contrast, about 1 in 10 $CD135^+$ DN1.1 cells from newborn mice were able to generate B lineage cells, indicating that the thymus at this age has about 300 of these precursors. Thus the thymus may indeed be colonised by precursors with T and B as well as NK and myeloid (see below) developmental potential. However, the number of these precursors in the adult is very low. Using thymus grafting experiments, Jotereau and colleagues [40, 41] had previously shown that the

Table 3

Cell-surface markers expressed by early thymocytes.

	c-kit CD117	FLT3 (CD135)	CD93	B220	IL-7Ra (CD127)	CD44	CD25
TSP	+++	+	ND	–	–	+++	–
DN1.1	+++	+	–	–	–	+++	–
DN1.2	+++	–	–	–	–	+++	–
DN2	+++	–	–	–	+	++	++
DN3	+	–	–	–	+	+/–	++

Table 4

Developmental potential of early thymocytes.

	B	T	Myeloid	NK
TSP	+	+++	++	++
DN1.1	–	+++	++	++
DN1.2	–	+++	++	++
DN2	–	+++	++	++
DN3	–	+++	–	–

perinatal mouse thymus was colonised by a wave of precursor cells. Our finding that these multi-lineage cells are much more abundant in the newborn mouse supports this idea. Table 3 summarises the cell surface markers used to distinguish early thymocyte subpopulations.

Recently we have shown that DN1 and 2 thymocytes require Notch, IL-7 and c-kit signalling for their continued T lineage differentiation [42, 43]. Moreover, we have provided evidence that expression of c-kit in DN1 and DN2 cells is under the direct control of Notch signalling [43]. DN1 and 2 cells are not committed to the T cell lineage since they can still efficiently give rise to NK and myeloid cells. For progression to T cell development, DN3 cells require Notch but not IL-7 and c-kit signalling. Even though growth of DN3 cells is still dependent on continued Notch signalling, this signalling is no longer capable of maintaining c-kit expression by DN3 cells. This probably means that Notch downstream signalling is altered at this important transition from DN2 to DN3 cells, a transition that also involves complete T cell commitment. Table 4 summarises the developmental potential of early thymocytes.

The advent of the OP9-DL1 culture system has revolutionised our understanding of early T cell development. The plating efficiency of this culture system for almost completely committed T cell progenitors approaches 100%. Using this culture system the phenotypic properties of progenitors with T cell developmental potential, as well as their growth requirements in terms of soluble factors and signalling molecules, can be studied in detail. EPLMs cultured under "B cell conditions" reconstitute the B cell compartment, whereas the same cells cultured under "T cell conditions" reconstitute the T cell compartment. The implications of being able to reconstitute either the B or T cell compartment from the same pool of EPLM are currently being explored.

Correspondence:
Antonius Rolink
Center for Biomedicine
Molecular and Developmental Immunology
Mattenstrasse 28
CH-4058 Basel
E-Mail: antonius.rolink@unibas.ch

References

1 Kondo M, Wagers AJ, Manz MG, Prohaska SS, Scherer DC, Beilhack GF, et al. Biology of hematopoietic stem cells and progenitors: implications for clinical application. Ann Rev Immunol. 2003;21:759–806.
2 Weissman IL. The road ended up at a stem cell. Immunological Reviews. 2002;185:159–74.
3 Abkowitz JL, Catlin S.N, McCallie MT, Guttorp P. Evidence that the number of hematopoietic stem cells per animal is conserved in mammals. Blood. 2002;100:2665–7.
4 Berenson RJ, Andrews RG, Bensinger WI, Kalamasz D, Knitter G, Buckner CD, Bernstein ID. Antigen CD34+ marrow cells engraft lethally irradiated baboons. J Clin Invest. 1988;81: 951–5.
5 Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol. 1984; 133:157–65.
6 Ogawa M. Changing phenotypes of hematopoietic stem cells. Exp Hematol. 2002;30:3–6.
7 Murray L, DiGiusto D, Chen B, Chen S, Combs J, Conti A, et al. Analysis of human hematopoietic stem cell populations. Blood Cells. 1994;20:364–9; discussion 369–70.
8 Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science. 1988;241:58–62.
9 Smith LG, Weissman IL, Heimfeld S. Clonal analysis of hematopoietic stem-cell differentiation in vivo. Proc Natl Acad Sci U S A. 1991;88:2788–92.
10 Kondo M, Weissman IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell. 1997;91:661–72.
11 Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature. 2000;404:193–7.
12 Born W, White J, Kappler J, Marrack P. Rearrangement of IgH genes in normal thymocyte development. J Immunol. 1988;140: 3228–32.
13 Balciunaite G, Ceredig R, Rolink AG. The earliest subpopulation of mouse thymocytes contains potent T, significant macrophage, and natural killer cell but no B-lymphocyte potential. Blood. 2005;105:1930–6.
14 Nutt SL, Heavey B, Rolink AG, Busslinger M. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. Nature. 1999;401:556–62.

15 Rolink AG, Nutt SL, Melchers F, Busslinger M. Long-term in vivo reconstitution of T-cell development by Pax5-deficient B-cell progenitors. Nature. 1999;401:603–6.
16 Schaniel C, Bruno L, Melchers F, Rolink AG. Multiple hematopoietic cell lineages develop in vivo from transplanted Pax5-deficient pre-B I-cell clones. Blood. 2002;99:472–8.
17 Mikkola I, Heavey B, Horcher M, Busslinger M. Reversion of B cell commitment upon loss of Pax5 expression. Science. 2002;297:110–3.
18 Zipori D. The nature of stem cells: state rather than entity. Nat Rev Genet. 2004;5:873–8.
19 Hochedlinger K, Jaenisch R. Monoclonal mice generated by nuclear transfer from mature B and T donor cells. Nature. 2002;415:1035–8.
20 Hochedlinger K, Jaenisch R. Nuclear reprogramming and pluripotency. Nature. 2006;441:1061–7.
21 Rolink AG. B-cell development and pre-B-1 cell plasticity in vitro. Methods Mol Biol. 2004;271:271–81.
22 Schmitt TM, Zuniga-Pflucker JC. Induction of T cell development from hematopoietic progenitor cells by delta-like-1 in vitro. Immunity. 2002;17:749–56.
23 Zuniga-Pflucker JC. T-cell development made simple. Nat Rev Immunol. 2004;4:67–72.
24 Adolfsson J, Mansson R, Buza-Vidas N, Hultquist A, Liuba K, Jensen CT, et al. Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. Cell. 2005;121: 295–306.
25 Katsura Y. Redefinition of lymphoid progenitors. Nature Reviews Immunology. 2002;2:127–32.
26 Nutt SL, Morrison AM, Dorfler P, Rolink A, Busslinger M. Identification of BSAP (Pax-5) target genes in early B-cell development by loss- and gain-of-function experiments. Embo J. 1998;17:2319–33.
27 Balciunaite G, Ceredig R, Massa S, Rolink AG. A B220+ CD117+ CD19- hematopoietic progenitor with potent lymphoid and myeloid developmental potential. Eur J Immunol. 2005;35:2019–30.
28 Ceredig R, Rauch M, Balciunaite G, Rolink AG. Increasing Flt3L availability alters composition of a novel bone marrow lymphoid progenitor compartment. Blood. 2006.
29 Ceredig R, Rolink T. A positive look at double-negative thymocytes. Nat Rev Immunol. 2002;2:888–97.
30 Fehling HJ, Gilfillan S, Ceredig R. Alpha beta/gamma delta lineage commitment in the thymus of normal and genetically manipulated mice. Adv Immunol. 1999;71:1–76.
31 Prinz I, Sansoni A, Kissenpfennig A, Ardouin L, Malissen M, Malissen B. Visualization of the earliest steps of gammadelta T cell development in the adult thymus. Nat Immunol. 2006;7: 995–1003.
32 Allman D, Sambandam A, Kim S, Miller JP, Pagan A, Well D, et al. Thymopoiesis independent of common lymphoid progenitors. Nat Immunol. 2003;4:168–74.
33 Benz C, Bleul CC. A multipotent precursor in the thymus maps to the branching point of the T versus B lineage decision. J Exp Med. 2005;202:21–31.
34 Harman BC, Jenkinson WE, Parnell SM, Rossi SW, Jenkinson EJ, Anderson G. T/B lineage choice occurs prior to intrathymic Notch signaling. Blood. 2005;106:886–92.
35 Porritt HE, Rumfelt LL, Tabrizifard S, Schmitt TM, Zuniga-Pflucker JC, Petrie HT. Heterogeneity among DN1 prothymocytes reveals multiple progenitors with different capacities to generate T cell and non-T cell lineages. Immunity. 2004;20: 735–45.
36 Radtke F, Wilson A, Stark G, Bauer M, van Meerwijk J, MacDonald H, et al. Deficient T cell fate specification in mice with an induced inactivation of Notch1. Immunity. 1999;10:547–58.
37 Sambandam A, Maillard I, Zediak VP, Xu L, Gerstein RM, Aster JC, et al. Notch signaling controls the generation and differentiation of early T lineage progenitors. Nat Immunol. 2005;6: 663–70.
38 Shortman K, Wu L. Early T lymphocyte progenitors. Annu Rev Immunol. 1996;14:29–47.
39 Zediak VP, Maillard I, Bhandoola A. Closer to the source: notch and the nature of thymus-settling cells. Immunity. 2005;23: 245–8.
40 Jotereau F, Heuze F, Salomon-Vie V, Gascan H. Cell kinetics in the fetal mouse thymus: precursor cell input, proliferation, and emigration. J Immunol. 1987;138:1026–30.
41 Jotereau FV, Le Douarin NM. Demonstration of a cyclic renewal of the lymphocyte precursor cells in the quail thymus during embryonic and perinatal life. J Immunol. 1982;129: 1869–77.
42 Balciunaite G, Ceredig R, Fehling HJ, Zuniga-Pflucker JC, Rolink AG. The role of Notch and IL-7 signaling in early thymocyte proliferation and differentiation. Eur J Immunol. 2005;35:1292–300.
43 Massa S, Balciunaite G, Ceredig R, Rolink AG. Critical role for c-kit (CD117) in T cell lineage commitment and early thymocyte development in vitro. Eur J Immunol. 2006;36:526–32.

Swiss
Medical Weekly

Official journal of
the Swiss Society of Infectious disease
the Swiss Society of Internal Medicine
the Swiss Respiratory Society

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses

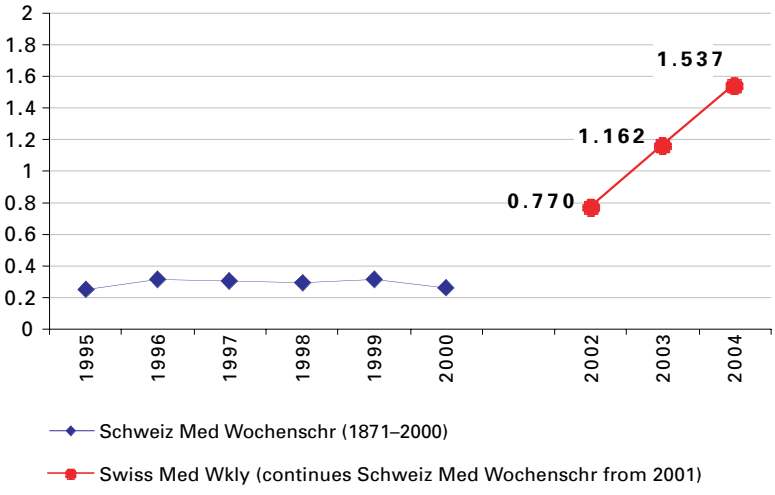
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

Impact factor Swiss Medical Weekly



EMH FMH Schwabe
Editores Medicorum Helveticorum

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts: submission@smw.ch
Letters to the editor: letters@smw.ch
Editorial Board: red@smw.ch
Internet: http://www.smw.ch