Michael Sieweke, Ph.D.

Directeur de Recherche

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German
Trilingual:
German, English, French

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Chemin des Ecureuils
13600 Ceyreste
France

PROFILE

Expert on stem cells, macrophage differentiation, hematopoietic and Maf family transcription factors. Principal research interests: Control of cell fate decisions and self renewal in stem cells and mature cells. Stability and plasticity of the differentiated phenotype. Role of macrophages in regeneration.

EDUCATION

1984-1986 **Eberhard-Karls Universiät, Tübingen, Germany,**
Undergraduate Program in Biochemistry

1986-1991 **University of California, Berkeley, USA,**
Ph.D. Program Biochemistry / Cell and Molecular Biology

1999 **Ruprecht-Karls Univeristät Heidelberg, Germany,**
Habilitation

APPOINTMENTS

1985-1986 **Friederich Miescher Labor, Max-Planck Insititut Tübingen, Germany,**
Research Assistant with *Dr. Walther Birchmeier*

1986-1991 **University of California, Berkeley, USA,**
Research Assistant and Ph.D student with *Dr. Mina Bissell*

1991-1995 **European Molecular Biology Laboratoty (EMBL), Germany**
Postdoctoral fellow with *Dr. Thomas Graf,* Differentiation Program

1996-1998 **European Molecular Biology Laboratoty (EMBL), Germany**
Staff Scientist-Junior Faculty, Cell Regulation and Developmental Biology Programs

1999- **Centre d'Immunologie de Marseille Luminy (CIML), France**
Group leader/ principal investigator

1999- **Centre National de la Recherche Scientifique (CNRS), France**
Directeur de Recherche (Associate Professor level)

AWARDS AND HONORS

1982: Best highschool diploma since foundation of school
1986-1987: Exchange scholar Eberhard-Karls-Universtät Tübingen/University of California, Berkeley (1-2 awards / year)
1987-1988: Stipend from the Department of Comparative Biochemistry, University of California, Berkeley
1988-1989: Fellowship of the Regents of the University of California
(most prestigious award of the university)
1989-1990: Julian D. Morgan Fellowship
awarded by the Regents of the University of California
1989-1990: Josephine de Karman Fellowship
1991-1992: Böhringer Ingelheim Fonds Fellowship
1992-1994: EMBO Fellowship
1999: Installation grant Communauté de Communes, Marseille Provence Métropole
1999-2001: ATIPE, French Young Investigator Award
2007- FRM group (prestigious label of Fondation pour la Recherche Médicale)

RESEARCH

Top 5 Publications

(original research, principal authorship only, abstracts attached)

Sieweke, M.H; et al.	***Science***, 248: 1656-1660 (1990)
Sieweke, M.H; et al.	***Cell,*** 85: 49-60, (1996)
Blanchi B.et al. and Sieweke M.H.	***Nature Neuroscience***, 6:109 (2003)
Sarrazin S, et al. and Sieweke M.H.	***Cell***, 138: 300-13 (2009)
Aziz A. et al. and Sieweke M.H	***Science***, in press (2009)

Major research contributions

Showed that TGFβ mediated extracellular matrix modulation and inflammation during wound healing contribute to a micro-environment favorable for tumorigenesis (1,2 and 4), a concept that was 15 years ahead of its time, as indicated by a series of prominent articles on inflammation and cancer in major impact journals over the last 5 years. [abstract 2 attached].

Discovered that the transcription factor MafB is required for the proper organization of neurons in the preBötzinger complex, a hindbrain region that generates the rhythm for respiratory movements. First identification of a transcription factor required for central respiratory rhythmogenesis, a finding with important implications for central respiratory disorders (19,23). [abstract and F1000 of 19 attached]

Showed the importance of transcription factor interactions, both repressive and cooperative, in hematopoietic lineage choice (6,8,10,11,13), which led to the development of the 'cocktail party' model (12), changed a purely DNA binding site oriented view to a concept of cross-antagonistic transcription factor function. [abstract 6 and 12 attached]

Discovered that the cross-antagonistic activity of the transcription factors MafB and PU.1 induces macrophage and dendritic cell fate respectively (15,22). [abstract and F1000 of 22 attached].

Showed that the transcription factor MafB forms an integrated circuit with the cytokine M-CSF in hematopoietic stem cells, where MafB is a threshold setter for M-CSF driven asymmetric myeloid commitment divisions (30). This finding resolved a long-standing debate about the capacity of cytokines to instruct cell fate and about the coordination of cell intrinsic and extrinsic regulators in lineage choice. The study has important implications for stem cell differentiation in general beyond the hematopoietic system [covered by N&V in 'Nature', 'Nature Reports Stem Cells' and 'Cell Stem Cell'; abstract and F1000 attached].

Discovered that MafB/cMaf deficient monocytes and macrophages can self-renew without loss of differentiated function by a cMyc/KLF4 dependent mechanism (31). This study shows that it is possible to uncouple terminal differentiation from cell cycle withdrawal and to amplify functional differentiated cells without stem cell intermediates. If non-tumorigenic self-renewal could be conferred to mature cells in general, the study may revolutionize the use of stem cells in regenerative medicine.
Furthermore MafB/cMaf deficient cells represent an extraordinary research tool to dissect monocyte/macrophage development and functions in inflammation, immunity and tissue repair and open up new perspectives for monocyte-based cellular therapies in infectious disease or tissue regeneration. [abstract attached]

Publications

1. Sieweke, M.H.; Stoker, A.W.; Bissell, M.J.; Evaluation of the cocarcinogenic effect of wounding in RSV-tumorgenesis; ***Cancer Research*****,** 49: 6419-6424 (1989).
2. Stoker, A.W. and Sieweke, M.H.;
   v-src induces clonal sarcomas and rapid metastasis following transduction with a replication defective retrovirus;
   Proc.Natl.Acad.Sci.USA, 86: 10123-10127 (1989).
3. Sieweke, M.H; Thompson, N.L.; Sporn, M.B.; Bissell, M.J.;
   Mediation of wound related Rous sarcoma virus tumorigenesis by TGFβ,
   Science, 248: 1656-1660 (1990).
4. Sieweke, M.H.; Bissell, M.J.;
   The Tumor-Promoting effect of wounding: A possible role for TGF-β- induced stromal alterations. ***Crit.Rev. Oncogenesis***, 5: 297-311 (1994).
5. Frampton, J.; McNagny, K.; Sieweke, M.H.; Philip, A.; Smith, G. andGraf, T.;
   v-Myb DNA binding is required to block thrombocytic differentiation of Myb-Ets-transformed multipoten haematopoietic progentiors.
   EMBO J. 14: 2866-2875 (1995)
6. Sieweke, M.H; Tekotte, H.; Frampton, J. and Graf, T.;
   MafB is an interaction partner and repressor of Ets-1 that inhibits erythroid differentiation.
   Cell 85: 49-60, (1996)
7. Eichmann, A.; Grapin-Botton, A.; Kelly, L.M.; Graf, T. Le Douarin, N.M. and Sieweke M.H.;
   The expression pattern of the mafB/kr gene in birds and mice reveals that the kreisler phenotype does not represent a null mutant.
   Mech. Development 65:111-122 (1997)
8. Sieweke, M.H; Tekotte, H.; Frampton, J. and Graf,T.; MafB represses erythroid genes and differentiation through direct interaction with Ets-1.
   Leukemia , 11, Suppl.3: 486-488 (1997)
9. Grapin-Botton A.; Bonnin M.-A.; Sieweke M.H.; and Le Douarin, N.M.;
   Environmental cues regulate kreisler expression in the rhombencephalon.
   Development 125: 1173-1181 (1998)
10. Sieweke, M.H.*; Tekotte, H. Jarosch, U. and Graf, T.;
    Cooperative interaction of Ets-1 with USF-1 on the distal enhancer of the HIV-1 LTR.
    EMBO J. 17: 1728-1739 (1998)
11. McNagny, K; Sieweke, M.H.; Döderlein, G.; Graf, T. and Nerlov, C.; Regulation of eosinophil-specific gene expression by a C/EBP-Ets complex and GATA-1.
    EMBO J. 17: 3669-3680 (1998)
12. Sieweke, M.H.* and Graf. T.
    A transcription factor party during blood cell differentiation
    Curr. Opin. Gen.& Dev. 8: 545-551 (1998)
13. Kim, W.Y.; Sieweke, M.H.*; Ogawa,E.; Englmeier, U.; Graf.T.and Ito,Y.
    Mutual activation of Ets-1 and AML1 DNA binding by direct interaction of their autoinhibitory domains
    EMBO J. 15:1609-1620 (1999)
14. Sieweke, M.H.
    Detection of transcription factor interactions with a yeast one hybrid screen.
    Methods Mol.Biol. 130: 59-77 (2000)

15. Kelly L.M., Englmeier U., Lafon I. Sieweke M.H*, and Graf T.
MafB is an inducer of monocytic differentiation
EMBO J. 19:1987-1997 (2000)
16. Posada R., Pettoello-Montovani M, Sieweke M.H., Graf, T. and Goldstein H.
Suppression of HIV-1 replication by a dominant negative Ets-1 mutant
AIDS Res. Hum. Retr., 16:1981-1989 (2000)
17. Ptak, K. Burnet, H., Blanchi, B.,,Sieweke, M., De Felipe C., Hunt, S.P., Monteau, R., and Hilaire, G.
The murine neurokinin NK_1 receptor gene contributes to the adult hypoxic facilitation of ventilation.
Eur. J. Neuroscience,12:2245-2252(2002)
18. Elvert G., Kappel A., Englmeier U., Lanz S., Acker T., Rauter M., Plate K., Sieweke M.H., Breier G. and Flamme I.
Synergistic interaction of hypoxia inducible factor (HIF)-2α and Ets-1 in the transcriptional activation of vascular endothelial growth factor receptor-2 (flk-1)
J.Biol.Chem., 278(9):7520-30. (2003)
19. Blanchi B., Kelly L.M. Viemari J.-C., Lafon,I., Burnet, H., Bévengut M., Tillmanns S., Daniel L., Graf T., Hilaire G.,and Sieweke M.H.
MafB deficiency causes defective respiratory rhythmogenesis and fatal central apnea at birth.
Nature Neuroscience, 6(10):1091-100 (2003)
20. Hilaire G, Burnet H, Ptak K, Sieweke M, Blanchi B, De Felipe C, Hunt S, Monteau R
Deletion of tachykinin NK1 receptor gene in mice does not alter respiratory network maturation but alters respiratory responses to hypoxia.
Adv Exp Med Biol. 536:497-504 (2003)
21. Sarrazin S., Sieweke, M.H.
The role of c-Myb in myeloid differentiation decisions
In « Myb Transcription Factors: Their Role in Growth, Differentiation and Disease »,Jon Frampton ed. (2004)
22. BakriY. ,Sarrazin S., Mayer U.P., Tillmanns S.,Boned A., Sieweke, M.H.
Induction of PU.1 in myeloid progenitors and monocytes specifies dendritic cell fate.
Blood 105, 2707-2716 (2005)
23. Blanchi B. and Sieweke, M.H.
Genetics of respiratory network development
Trends Molec. Medicine, 11(1):23-30 (2005)
24. Aziz A, Vanhille L, Mohideen P, Kelly LM, Otto C, Bakri Y, Mossadegh N, Sarrazin S, Sieweke MH.
Development of macrophages with altered actin organization in the absence of MafB.
Mol Cell Biol 26(18):6808-18 (2006)
25. Artner, I., Blanchi,B., Raum, J.C., Guo, M., Kaneko, R., Cordes, S., Sieweke, M.H. and Stein, R.
MafB is required for islet β cell maturation
Proc.Natl.Acad.Sci.USA 104(10):3853-8 (2007)
26. Tillmanns, S., Otto.,C. Jaffray,E., Bakri,Y., DuRoure, C.,VanHille L., Sarrazin, S., Hay,R.T., Sieweke, M.H.
SUMO-modification regulates MafB driven macrophage differentiation by enabling Myb dependent transcriptional repression
Mol Cell Biol 27(15):5554-64 (2007)

27. Lambert, C., Vanhille L., Zou, P, Sieweke, M.H. and Wilmanns, M.
Regulation of the transcription factor Ets-1 by DNA-mediated homo-dimerization
EMBO J. 27(14):2006-17 (2008)
28. Auffray, C., Sieweke, M.H., Geissmann, F.
Blood monocytes: development, heterogeneity, relationship with dendritic cells
Annual Rev. Immunol., 27:669-92 (2009)
29. Boukarabila H, Saurin AJ., Batsché E, Mossadegh N, van Lohuizen M, Otte AP, Pradel J§, Muchardt C, Sieweke MH§/Duprez E§
The PRC1 Polycomb group complex interacts with PLZF/RARA to mediate leukemic transformation
Genes & Dev, 23(10):1195-206 (2009)
30. Sarrazin S, Mossadegh-Keller N, Fukao T, Aziz A, Mourcin F., Kelly L, Vanhille L, Kastner P, Chan S, Duprez E, Otto, C, Sieweke MH
MafB restricts M-CSF dependent myeloid commitment divisions of hematopoietic stem cells
Cell, 138(2):300-13 (2009)
31. Aziz A, Soucie E, Sarrazin S, Sieweke MH
MafB/c-Maf deficiency enables self-renewalof differentiated functional macrophages
Science, in press (2009)
32. Geissmann F, Manz M, Jung S, Sieweke M, Merad M, Ley K
Development of monocytes, macrophages and dendritic cells
Science, in revision (2009)

§ joint last author, * corresponding author, lab members underlined

F1000 evaluations (attached)

1. Blanchi B, et.al. Nat Neurosci 6:1091-100 (2003) F1000 factor: 6.0
http://www.facultyof1000.com/article/14513037/evaluation
2. Bakri, Y. et al., Blood 105: 2707-2716 (2005) F1000 factor: 3.0
http://f1000biology.com/guardpages/evaluation/1023616//article
3. Sarrazin, S. et al., CELL138(2):300-13 (2009) F1000 factor: 6.0
http://www.f1000biology.com/article/id/1163793

Patents

1. European patent EP 07 300717.1.-2405: Method for generating, maintaining and expanding monocytes, and/or macrophages and/or dendritic cells in long term culture. filed 01/2007
2. United States patent PCT/EP08/50221: Method for generating, maintaining and expanding monocytes, and/or macrophages and/or dendritic cells in long term culture. filed 07/2008
3. European patent EP 09224: A method for inducing extended self-renewal of functionally differentiated somatic cells . filed 07/2009

Invited Lectures *(since 2000)**:***

2000
- Schering-Plough, Lyon, *France, 17.1.2000*
- Developmental Biology Institute Marseille, Marseille, *France, 13.10.2000*
- Centre de Génétique Moléculaire et Cellulaire, Lyon, *France, 27.10. 2000*
- EMBL-Hamburg, Hamburg, *Germany, 17.11.2000*
- Institut Curie, Paris, *France, 22.12.2000*

2001
- INSERM U372, Marseille, *France, 20.3.2001*
- Institute de Biologie de Lille , Lille, *France, 16.11.2001*

2002
- Mouse Molecular Genetics Conference, Cold Spring Harbor, *USA, 29.8.2002*
- EMBL-Hamburg, Hambur*g, Germany 28.03.2002*
- 12th Meeting of the International Society of Differentiation, Lyon, *France, 15.9.2002*
- 9ème congrès de Club Hématopoïèse et oncogenèse, *France, 14.10.2002*

2003
- IXth Oxford Conference on Modelling and control of Breathing, Paris, *France,15.9.2003*
- MRC, Clinical Sciences Centre, Faculty of Medicine, Imperial College, London,*UK, 12.02. 2003*

2004
- International Congress of Biochemistry, Marakech, *Marocco, 5.5.2004*
- University of Michigan School of Medicine, Ann Arbor, MI, *USA,27.07.2004*
- Conference Luminy Bio 3, Marseille, *France, 4.10.2004*
- SYMPOSIUM 'CANCER ,TRANSCRIPTION AND DEVELOPMENT, Barcelona, *Spain, 16.11.2004*

2005
- 4th International Symposium of the Volkswagen Foundation Heidelberg, *Germany*, *7.10.2005*

2006
- Albert Einstein College of Medicine, New York, NY, *USA, 3.7.2006*
- Hopital Necker, Paris, *France 14.12.2006*
- Université de Bourgogne, Dijon, *France, 19.12.2006*

2007
- Keystone Symposium'The Macrophage: Homeostasis, Immunoregulation and Disease', Copper Mountain Resort, Copper Mountain, *USA, 13.4.2007*
- The Biomedical Research Centre, University of British Columbia, Vancouver, *Canada, 17.4.2007*
- Fondation Schlumberger conference: 'Dendritic cell biology' Fondation des Treilles, Les Treilles, *France, 5.6.2007*
- Institute of Molecular and Cell Biology, Biopolis, *Singapore, 25.7.2007*

2008
- Californmia-France Stem Cell Initiative, Marseille, *France, 25.9.2008*
- Institut Pasteur, Paris, *France, 7.11.2008*
- Molecular Hematopoiesis Conference, London, *UK*, *10.11.2008*
- King's College, London, *UK, 11.11.2008*

2009
- Fondation Schlumberger conference: 'Monocyte, Macrophage and Dendritic cell biology' Fondation des Treilles, Les Treilles, *France, 3.3.2009*
- Institut Pasteur Korea, Seoul, *South Korea, 18.5.2009*
- ISSCR 7th Annual Meeting of the International Society of Stem Cell Research Barcelona, *Spain, 2.7.2009*
- Institut Curie, Paris, *France, 25.9.2009*
- 16ème Congrés du Club Hématopoïèse et Oncogénèse, *France, 2.10.2009*
- 'Reprogramming Cell Fate : Basic Biology and Medical Perspectives' European School of Molecular Medicine, Milan, *Italy*, *11.12.2009*

2010
- Immunity in context -Development and Survival Signals in the Immune System, Weizmann Institute of Science, Rehovot, *Israel, 1.3.2010*
- Oxford University, Oxford, *UK*, *16.2.2010*
- Symposium in honor of Mina Bissell, UC Berkeley, Berkeley, *USA, 8.5.210*

TEACHING

Courses

- "Transcription factors in oncogenesis and differentiation", Graduate lab course and lectures Universität Heidelberg and EMBL, 1996-1998
- "A brief history of oncogenes", Lecture, graduate course, EMBL 1996-1997
- "Nuclear factors in cell fate decisions" Lecture, graduate course, 1998-1999
- "Methods in Molecular Biology", undergraduate lab course, Universität Heidelberg, 1997-1999
- "Methods in Molecular Biology/ DNA", Graduate lab course, Universität Heidelberg, 1997
- "Methods in Molecular Biology/ protein biochemistry", Graduate lab course, Universität Heidelberg, 1997-1999
- " A transcription factor party during blood cell differentiation" Lecture, Masters program "Cellules souches, plasticité cellulaire et développement embryonnaire précoce", Ecole Normale Supérieure, Lyon, 2001
- 'Differentiation and Growth control: Molecular mechanisms of leukemogenesis' Seminar, International Masters Program, Universität Heidelberg, 2002-2008
- 'Differentiation and Growth control: Differentiation decisions of lymphoid and hematopoietic cells', Seminar, International Masters Program, Universität Heidelberg, 2002-2008
- 'Transcription factor function in blood cell differentiation' Lecture and workshop, Masters Program 'Genes, Cellules et Development' Université Toulouse, 2004
- 'Development of immune cells' Lecture and workshop, LACI winterschool of advanced Immunology, CIML, Marseille, 2007
- 'Stem Cells', lecture, Development and Immunology Masters Program, Université de Aix-Marseille, 2009

Teaching organization

- 2000-2005 Head of thesis advisory committee at CIML
- Jury Member in 15 thesis and 1 habilitation exams

Training and mentoring

Students

(year of PhD and current position if applicable)

- M. Weigand MD 1997, MCU in research hospital, Erlangen, Germany
- L.Kelly, PhD 2000, Head of research unit, Boehringer Ingelheim, USA
- U. Mayer, Master 1999, PhD 2004, Biotech marketing, Serotec, Germany
- S. Tillmanns, PhD, June 2004 Postdoc Universität Freiburg, Germany
- B. Blanchi, PhD 2004**,** Postdoc UCLA, USA
- A. Aziz, PhD 2008, Postdoc Cambridge, UK
- L. VanHille, PhD 2009**,** Postdoc IMCB, Biopolis, Singapore
- H. Boukarabila, PhD 2009
- F. Imperatore, present
- Q. Lahmar, present
- M. Beniazza, present

8 DEA/masters/Diploma students, over a dozen summer or internship students training

Postdocs

(year s in the lab and current position if applicable)

- Y. Bakri 2001-2004, Associate Professor, University of Rabat, Marocco
- L. Signon 2001-2002, CR2 staff scientist, IBSM, Marseille, France
- S. Sarrazin 2002-2006, CR2 staff scientist in the lab
- T. Fukao 2002-2004, Group leader, Max-Planck-Institute Freiburg, Germany
- P. Mohedeen 2002-2003 Research Scientist, King Faisal Hospital, Saudi Arabia
- C. Otto: 2005-2008, CEO, marketing startup, France
- F. Zelada: 2006 - present
- E. Soucie: 2006 – present

Senior researchers

(year s in the lab and current position if applicable)

- Estelle Duprez, CR1 staff scientist, 2002-2009 Group leader, IPC, Marseille, France
- Sandrine Sarrazin, CR2 staff scientist, 2006-present
- Carole Pouyet, MCU lecturer, 2008-present

Transfer of Scientific Knowledge to the Public

- “Vom Genotyp zum Phänotyp: Entwicklungsbiologie heute” Training course for high school teachers in the “Gen-Welten” exhibition of the “Landesmuseum für Technik und Arbeit”, Mannheim, Germany,1998
- National and international media coverage on Blanchi et al., Nature Neurosc.2003: Dépeche AFP, coverage in LeFigaro, L’Est Republican, Reader's Digest, La Recherche, Radio France Info, TV M6 (selection)
- Media coverage on Sarrazin et al. Cell 2009: Nouvel observateur and others
- Fête de Science 2009: lecture on stem cells to highschool students

COMISSIONS AND RESEARCH EVALUATION

- Member Commission Nationale 1, ARC (Association pour la Recherche contre le Cancer), 2002- 2008
- Member of INSERM unit evaluation committee , 2006
- Evaluation of INSERM reseacher applications for promotion, 2007
- Member of AERES committee for institute unit evaluation, 2010

ad hoc reviewer for:
- Mildred Scheel Foundation (German Cancer Research Foundation)
- ACI (French Ministry of Research),
- LNCC, La Ligue Nationale contre le Cancer
- SFH, Societe Francaise de la Hematologie
- FRM, Fondation pour la Recherche Medicale
- Minerva Program Israel/Germany,
- InCA, Institut National Du Cancer
- Wellcome Trust
- Cancer Research UK

MEMBERSHIPS

- International Society for Stem Cell Research (ISSCR)
- American Society for the Advancement of Science (AAAS)
- Société Française de l'Hématologie (SFH)

CONSULTING

- LG Pharmaceuticals Korea and Institute Pasteur Korea, Seoul, South Korea
  on 'Leukemic Stem cells' , 2004-2005

COMPETIVELY OBTAINED FUNDING (selected)

2004 Fondation de France (French Charity)
ACI (French Ministry of Research and Technology)
ARC (French Cancer Charity)
Total 2004: 439 000 Euros

2005 AICR (British Cancer Charity)
Volkswagen Foundation (German Research Foundation)
Total 2005: 478 000 Euros

2006 ARC (French Cancer Charity)
Total 2006: 125 000 Euros

2007 Group label FRM (French Charity)
ANR (National French Research Agency)
INCA (French Cancer Research Agency)
Total 2007: 1 023 000 Euros

2008 Agenge Biomedicine (French Agency for transplantation Medicine)
ARC (French Cancer Charity)
Total 2008: 306 000 Euros

Average last 5 years: 474 000 Euros / year

sphingoids (*9, 24*). The plasma membrane is a major site of sphingosine localization; it contains sphingomyelinase activity that could generate sphingosine from endogenous substrates (*25*), and it may be the primary site of inhibition of PKC. However, the solubility of sphingosine (>10 μM under intracellular conditions) permits its movement within the cytosol to internal membranes. We propose that at the ER membrane, sphingosine is converted most probably to sphingosine-1-phosphate and there mediates Ca^{2+} release. Analyses of sphingosine-1-kinase in platelets suggest that the enzyme is cytosolic, although with multiple forms (*22, 26*); a microsomal enzyme exists in *Tetrahymena pyriformis* (*27*), a location also suggested by our studies (*20*). Sphingosine-1-phosphate is curiously insoluble in either aqueous or nonaqueous solvents (*28, 29*); thus, if sphingosine-1-phosphate is formed in the ER membrane, it could stay trapped there. Sphingosine-1-phosphate lyase, an enzyme cleaving sphingosine-1-phosphate to palmitaldehyde and phosphoethanolamine, is a known microsomal enzyme (*28*). Thus, the ER appears to have the means to form, retain, and degrade sphingosine-1-phosphate. The regulation of either enzymic step could control Ca^{2+} permeability and hence Ca^{2+} signaling in cells.

REFERENCES AND NOTES

1. M. J. Berridge and R. F. Irvine, *Nature* **341**, 197 (1989).
2. D. L. Gill, *ibid.* **342**, 16 (1989); T. Furuichi *et al.*, *ibid.*, p. 32; C. D. Ferris, R. L. Huganir, S. Supattapone, S. H. Snyder, *ibid.*, p. 87; G. A. Mignery, T. C. Südhof, K. Takei, P. De Camilli, *ibid.*, p. 192.
3. Y. Nishizuka, *Science* **233**, 305 (1986).
4. P. Needleman *et al.*, *Annu. Rev. Biochem.* **55**, 69 (1986); B. Samuelsson *et al.*, *Science* **237**, 1171 (1987).
5. Y. A. Hannun and R. M. Bell, *Science* **243**, 500 (1989).
6. A. H. Merrill, Jr., and V. L. Stevens, *Biochim. Biophys. Acta* **1010**, 131 (1989).
7. Y. A. Hannun, C. R. Loomis, A. H. Merrill, Jr., R. M. Bell, *J. Biol. Chem.* **261**, 12604 (1986); A. H. Merrill, Jr., *et al.*, *ibid.*, p. 12610 (1986); Y. A. Hannun, C. S. Greenberg, R. M. Bell, *ibid.* **262**, 13620 (1987); M. Faucher, N. Gironès, Y. A. Hannun, R. M. Bell, R. J. Davis, *ibid.* **263**, 5319 (1988).
8. Y. A. Hannun and R. M. Bell, *Science* **235**, 670 (1987).
9. E. Wilson *et al.*, *J. Biol. Chem.* **263**, 9304 (1988).
10. A. B. Jefferson and H. Schulman, *ibid.*, p. 15241.
11. D. L. Gill and S. H. Chueh, *ibid.* **260**, 9289 (1985); T. Ueda, S. H. Chueh, M. W. Noel, D. L. Gill, *ibid.* **261**, 3184 (1986); S. H. Chueh and D. L. Gill, *ibid.*, p. 13883.
12. D. L. Gill, T. Ueda, S. H. Chueh, M. W. Noel, *Nature* **320**, 461 (1986).
13. J. S. Norris *et al.*, *ibid.* **248**, 422 (1974).
14. A. H. Merrill, Jr., *et al.*, *Biochemistry* **28**, 3138 (1989).
15. S. H. Chueh, J. M. Mullaney, T. K. Ghosh, A. L. Zachary, D. L. Gill, *J. Biol. Chem.* **262**, 13857 (1987); J. M. Mullaney, S. H. Chueh, T. K. Ghosh, D. L. Gill, *ibid.*, p. 13865.
16. J. M. Mullaney, M. Yu, T. K. Ghosh, D. L. Gill, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2499 (1988).
17. T. K. Ghosh, J. M. Mullaney, F. I. Tarazi, D. L. Gill, *Nature* **340**, 236 (1989); D. L. Gill, T. K. Ghosh, J. M. Mullaney, *Cell Calcium* **10**, 363 (1989).
18. Our previous studies revealed this third pool of Ca^{2+}, which is unresponsive to IP_3 and GTP (*15*). GTP is believed to permit connections and transfer of Ca^{2+} between the IP_3-sensitive and IP_3-insensitive Ca^{2+} pools, permitting IP_3 to release Ca^{2+} from both pools (*16, 17*). Thus, in the presence of both IP_3 and GTP, the two interconnected pools are empty.
19. T. K. Ghosh, P. S. Eis, J. M. Mullaney, C. L. Ebert, D. L. Gill, *J. Biol. Chem.* **263**, 11075 (1988).
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Mediation of Wound-Related Rous Sarcoma Virus Tumorigenesis by TGF-β

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In Rous sarcoma virus (RSV)–infected chickens, wounding leads to tumor formation with nearly 100% frequency in tissues that would otherwise remain tumor-free. Identifying molecular mediators of this phenomenon should yield important clues to the mechanisms involved in RSV tumorigenesis. Immunohistochemical staining showed that TGF-β is present locally shortly after wounding, but not in unwounded controls. In addition, subcutaneous administration of recombinant transforming growth factor–β1 (TGF-β1) could substitute completely for wounding in tumor induction. A treatment protocol of four doses of 800 nanograms of TGF-β resulted in v-*src*–expressing tumors with 100% frequency; four doses of only 10 nanograms still led to tumor formation in 80% of the animals. This effect was specific, as other growth factors with suggested roles in wound healing did not elicit the same response. Epidermal growth factor (EGF) or TGF-α had no effect, and platelet-derived growth factor (PDGF) or insulin-like growth factor–1 (IGF-1) yielded only occasional tumors after longer latency. TGF-β release during the wound-healing response may thus be a critical event that creates a conducive environment for RSV tumorigenesis and may act as a cofactor for transformation in this system.

ROUS SARCOMA VIRUS (RSV) WAS the first RNA tumor virus to be discovered (*1*). RSV rapidly transforms many cell types in culture (*2*), but its ability to induce tumors in vivo is highly dependent on the tissue environment (*3–5*). An example of this is seen in young chicken hatchlings, in which a sarcoma is rapidly formed only at the site of virus injection and at the site of experimentally induced wounds (*5, 6*). In spite of circulating infectious virus, other tissues stay generally free of tumors during the early stages of pathogenesis (*5, 6*). Thus the infliction of a wound and the subsequent healing process appear to confer a state conducive to RSV tumorigenesis. We have used this model system to identify factors that contribute to the generation of such a competent environment.

Growth factors are important in tissue reorganization during the wound-healing process (*7*) and have been implicated in the sustained growth of neoplasms. They may therefore be mediators in the creation of the competent environment. TGF-β has been shown to be one of the most potent effectors of the wound-healing reaction. It enhances

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MafB Is an Interaction Partner and Repressor of Ets-1 That Inhibits Erythroid Differentiation

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Summary

Using a yeast one-hybrid screen with a DNA-bound Ets-1 protein, we have identified MafB, an AP-1 like protein, as a direct interaction partner. MafB is specifically expressed in myelomonocytic cells and binds to the DNA-binding domain of Ets-1 via its basic region or leucine–zipper domain. Furthermore, it represses Ets-1 transactivation of synthetic promoters containing Ets binding sites and inhibits Ets-1–mediated transactivation of the transferrin receptor, which is known to be essential for erythroid differentiation. Accordingly, overexpression of MafB in an erythroblast cell line down-regulates the endogenous transferrin receptor gene and inhibits differentiation without affecting cell proliferation. These results highlight the importance of inhibitory interactions between transcription factors in regulating lineage-specific gene expression.

Introduction

The phenotype of a differentiated cell is ultimately determined by the set of its active genes. Since the execution of a particular gene-expression program is only rarely triggered by a single "master gene," it has been proposed that this usually requires the combinatorial action of several transcription factors (Ness and Engel, 1994; Orkin, 1995). It has been assumed that this is due to the simple additive effect of the factors present, and also that it involves protein–protein interactions that can further modify the activities of the individual partners and thus increase the options for activation of diverse genetic programs. Such regulatory interactions with other factors are well described for the Ets proteins, a family of transcription factors containing a characteristic helix–turn–helix DNA-binding domain (Janknecht and Nordheim, 1993; Treisman, 1994). For example, complex formation of the Ets protein Elk-1/SAP-1 with serum response factor dimers is critical for regulation of the c-*fos* promoter (Dalton and Treisman, 1992); the Ets-related protein GABPα forms heterotetramers with GABPβ to activate immediate early promoters of HSV-1 (LaMarco et al., 1991); and the association of the Ets family member Pu.1/Spi-1 with NF-EM5/Pip1 is important for the regulation of immunoglobulin light chain enhancers (Eisenbeis et al., 1995).

Much less is known about protein partners of Ets-1, the founding member of the family. Ets-1 occurs in two alternatively spliced isoforms, p54ets-1 and p68^{ets-1}, which differ in their N-terminal transactivation domain. p68^{ets-1} is of particular interest because it represents the cellular homolog of the v-Ets oncoprotein, which is encoded as a fusion protein with v-Myb by the E26 leukemia virus (Leprince et al., 1983; Nunn et al., 1983). Within the hematopoietic system, v-Ets appears predominantly to affect the growth and differentiation of erythroid cells. Thus, it transforms erythroid cells and enhances the erythroid transformation potential of v-ErbA and v-Myb (Metz and Graf, 1991, 1992). Ets-1 is expressed in erythroblasts transformed by the v-*erbA* and v-*erbB*-containing avian erythroblastosis virus (Ghysdael, 1986) and in erythroblasts of early yolk sac of the chick embryo (Quéva et al., 1993). Together with the finding that Ets-1 is capable of activating promoters of erythroid genes (Seth, 1993; J. F. et al., unpublished data), these data suggest that it plays a role in early erythroid differentiation.

It is unknown whether Ets-1 activity is regulated by direct interactions with other transcription factors in hematopoietic cells. In other systems, Ets-1 has been shown to cooperate with various transcriptional activators in regulating gene activity (Gégonne et al., 1993; Giese et al., 1995), such as with the basic region–leucine zipper (b–Zip) AP-1 family (Wasylyk et al., 1990). In addition, a number of promoters contain functionally important AP-1 and Ets–responsive elements in close proximity (Gutman and Wasylyk, 1990; Nerlov et al., 1991; Wasylyk et al., 1991). It has also been shown that the Drosophila Ets homolog *pointed* and the AP-1 protein D-Jun both lie at the endpoint of the Ras/MAP kinase signal transduction pathway (Bohmann et al., 1994; Brunner et al., 1994; O'Neill et al., 1994) and cooperate in the induction of R7 photoreceptor cells (Treier et al., 1995). AP-1–like factors have recently also been implicated in erythroid differentiation. Thus, several erythroid promoters have been shown to contain functionally relevant binding sites with a core AP-1 recognition sequence (Higgs and Wood, 1993). These sites are recognized by the heterodimeric transcription factor NF-E2, which consists of two bZip-type factors, a hematopoietic-specific 45 kDa subunit (Andrews et al., 1993a; Ney et al., 1993) and a broadly expressed 18 kDa subunit that belongs to the AP-1–related Maf family of transcription factors (Andrews et al., 1993b; Igarashi et al., 1994).

In the present study, we have searched for direct protein interaction partners of Ets-1 that might modify its activity, using a yeast one-hybrid screen. We have identified MafB, a bZip protein of the Maf family, as such a partner. This protein is highly expressed in myelomonocytic but not in erythroid cells. Its overexpression in erythroblasts inhibits transactivation of the transferrin receptor gene by Ets-1 and represses erythroid differentiation. These results demonstrate a functional interaction between members of the Ets and Maf protein families in the hematopoietic system and suggest that the repression of Ets-1 by MafB plays a role in the regulation of lineage-specific gene expression.

A transcription factor party during blood cell differentiation

Michael H Sieweke* and Thomas Graf†

Recent studies have shown that hematopoietic transcription factors can engage in multiple protein–protein interactions. Accumulating evidence indicates that specific complexes define differentiation lineages and differentiation stages. It is proposed that these complexes acquire new functions during blood cell differentiation through successive changes in composition – much as discussion topics of groups at a cocktail party take new directions as new people join and others leave.

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Abbreviations
ES embryonic stem
HLH helix-loop-helix
MEPs multipotent progenitor cells

Introduction

The hematopoietic system is a paradigm for the development of different specialized cell types from multipotent progenitors. How specific gene expression programs are selected and maintained is a major focus of investigation. The central role of transcription factors in these processes has been highlighted by gene-inactivation studies, promoter analysis, and ectopic expression of lineage-restricted factors [1–3]. Several recent reviews focus on the transcription factors important for the specification of the myeloid [4], lymphoid [5,6] and erythroid [7] lineages.

Rather than being controlled by single master regulators, lineage-specific gene expression appears to depend on the combination of factors in overlapping expression domains [8]. The complex arrays of transcription factor binding sites present in tissue-specific enhancer/promoter regions are generally thought to recruit subsets of binding activities from the complement of factors expressed in a particular cell type [9]. Based on models of bacterial gene regulation, it has been proposed that protein interactions between the individual enhancer-bound factors serve to promote cooperative assembly on the DNA ([10] and references therein). Recent observations, however, demonstrate that major functions of typical transcription factors do not require DNA binding (for example, see [11••]), suggesting that the protein interaction potential of transcription factors carries information beyond the stabilization of DNA contacts.

On the basis of recent studies in the hematopoietic system, we explore the view that multiple but selective interactions between transcription factors reflect different functions of the same protein that may thus be a defining element for the execution of distinct gene-expression programs. We propose that transcription factors can assemble independently of an enhancer template into multicomponent complexes with distinct activities on different enhancers. Furthermore, we suggest that differentiation is driven by the successive exchange of individual components of the protein complexes. This model resembles the dynamics of a cocktail party where the composition and themes of individual conversation groups change with the stepwise exchange of participants.

To illustrate these concepts, we have selected examples from the erythroid and myeloid compartment of the hematopoietic system, focusing on heterotypic transcription factor interactions. We do not discuss the known dimerization potentials of classic leucine zipper (bZip), helix-loop-helix (HLH) or nuclear hormone receptor-type transcription factors.

Erythroid connections

The analysis of transcription factor complexes in the erythroid/megakaryocytic compartment suggests that their composition changes as differentiation proceeds from an early hematopoietic progenitor to either an erythroid or a megakaryocytic cell (summarized in Figure 1). A transcription factor that participates in several of these complexes is the zinc-finger protein GATA-1. For example, it was found to form a complex with the LIM domain protein LMO-2 in erythroid cells [12]. The HLH protein SCL (tal-1) also interacts with LMO-2 but not with GATA-1, suggesting that LMO-2 acts as a bridging factor between SCL and GATA-1 [12]. Other than GATA-1, LMO-2, SCL and its heterodimerization partner E47, the complex appears to contain at least one more protein named Ldb-1 [13••]. The functional relevance of the complex was underscored by binding-site selection studies: complexes precipitated by antisera against various components bound to a specific composite GATA and E-box element (the binding site for HLH proteins). It still needs to be demonstrated, however, that such binding sites play a role in authentic erythroid promoters/enhancers.

What is the biological significance of the GATA-1/LMO-2/SCL protein complex for erythroid differentiation? Some indications come from gene-inactivation studies: Mice lacking SCL or LMO-2 show very similar phenotypes

nature neuroscience

MafB deficiency causes defective respiratory rhythmogenesis and fatal central apnea at birth

Bruno Blanchi[1,6], Louise M Kelly[1,5,6], Jean-Charles Viemari[2,6], Isabelle Lafon[1], Henri Burnet[2], Michelle Bévengut[2], Silke Tillmanns[1], Laurent Daniel[3], Thomas Graf[4], Gerard Hilaire[2] & Michael H Sieweke[1]

The genetic basis for the development of brainstem neurons that generate respiratory rhythm is unknown. Here we show that mice deficient for the transcription factor MafB die from central apnea at birth and are defective for respiratory rhythmogenesis *in vitro*. MafB is expressed in a subpopulation of neurons in the preBötzinger complex (preBötC), a putative principal site of rhythmogenesis. Brainstems from *Mafb*$^{-/-}$ mice are insensitive to preBötC electrolytic lesion or stimulation and modulation of rhythmogenesis by hypoxia or peptidergic input. Furthermore, in *Mafb*$^{-/-}$ mice the preBötC, but not major neuromodulatory groups, presents severe anatomical defects with loss of cellularity. Our results show an essential role of MafB in central respiratory control, possibly involving the specification of rhythmogenic preBötC neurons.

Breathing in mammals is a vital and continuous behavior that is controlled by a network of brainstem neurons. Physiological and anatomical studies have identified several groups of respiratory neurons in the pons and medulla of the brainstem that generate respiratory rhythm or provide regulatory input[1–3]. This includes a network in the ventrolateral part of the medulla that is essential for rhythmogenesis[4–8]. Cooperation between inspiratory neurons in the preBötC and preinspiratory (pre-I) neurons inside and rostral to this structure[9,10] has been shown to be important for this[9,11–13].Within this network, the preBötC is a major contributor to respiratory rhythmogenesis *in vitro*[12,13] and *in vivo*[14–17].

Despite these advances, the genetic and cellular basis that underlies rhythmogenesis has not been determined. Some knockout mutations affect regulatory elements of the respiratory network[18–23], but genes specifying the development and identity of critical rhythm-generating neurons in the brainstem have so far remained elusive. Here we have identified the transcription factor MafB as a marker of a subpopulation of preBötC neurons and show that it is required for normal preBötC development and function.

MafB is a bZIP transcription factor that is a crucial determinant of differentiation decisions in the hematopoietic system[24,25], of podocyte differentiation[26] and of rhombomere specification in the early hindbrain[27]. An X-ray-induced mouse mutation called *kreisler*[28], which affects the *Mafb* gene, specifically abolishes MafB expression in the early hindbrain but leaves the coding region intact and does not affect other expression sites[27,29]. To study the *in vivo* function of MafB in other tissues, we inactivated the gene by homologous recombination. Whereas homozygous *kreisler* mice have a normal respiratory frequency and live to adulthood[28,30], we show here that *Mafb*$^{-/-}$ mice died from respiratory arrest at birth, were defective in generating respiratory-related rhythm *in vitro* and had a severe anatomical and functional defect in the preBötC.

RESULTS

Fatal breathing failure of *Mafb*$^{-/-}$ mice at birth

To inactivate the *Mafb* gene, we replaced the single-exon coding sequence by homologous recombination in R1 embryonic stem cells (**Fig. 1a**). We obtained several highly chimeric mice that transmitted the allele to the germ line as determined by Southern blotting (**Fig. 1b**). Absence of MafB expression in knockout animals was verified by western and northern blot analysis of *in vitro*–generated *Mafb*$^{-/-}$ macrophages, a cell type that expresses high amounts of MafB (**Fig. 1c** and data not shown). Crossing of *Mafb*$^{+/-}$ mice did not give rise to live offspring with a *Mafb*$^{-/-}$ genotype.

Analysis of embryos, however, showed a normal Mendelian ratio of genotypes up to embryonic day (E)18.5, just before birth, indicating that lethality occurred at birth (**Fig. 2a**). Close observation of pregnant mice at delivery showed that *Mafb*$^{-/-}$ pups were born normally but suffered from gasping behavior and cyanosis and died within the first 2 h after birth (**Fig. 2b**). To analyze whether this phenotype was due to breathing anomalies, we surgically delivered E18.5 embryos and analyzed respiratory activity by recording breathing-associated pressure changes (plethysmography). Whereas *Mafb*$^{+/+}$ (n = 12) and *Mafb*$^{+/-}$ (n = 28) mice initiated normal breathing behavior within a few minutes, *Mafb*$^{-/-}$ (n = 9) animals did not and ventilated essentially by intermittent deep gasping

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Blanchi B, Kelly LM, Viemari JC, Lafon I, Burnet H, Bévengut M, Tillmanns S, Daniel L, Graf T, Hilaire G, Sieweke MH

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Balance of MafB and PU.1 specifies alternative macrophage or dendritic cell fate

Youssef Bakri, Sandrine Sarrazin, Ulrich P. Mayer, Silke Tillmanns, Claus Nerlov, Annie Boned, and Michael H. Sieweke

Macrophages and myeloid dendritic cells (DCs) represent alternative differentiation options of bone marrow progenitors and blood monocytes. This choice profoundly influences the immune response under normal and pathological conditions, but the underlying transcriptional events remain unresolved. Here, we show that experimental activation of the transcription factors PU.1 and MafB in transformed chicken myeloid progenitors triggered alternative DC or macrophage fate, respectively. PU.1 activation also was instructive for DC fate in the absence of cytokines in human HL-60 cell-derived myeloid progenitor and monocyte clones. Differentiation of normal human monocytes to DCs led to a rapid increase of PU.1 to high levels that preceded phenotypic changes, but no MafB expression, whereas monocyte-derived macrophages expressed MafB and only moderate levels of PU.1. DCs inducing levels of PU.1 inhibited MafB expression in monocytes, which appeared to be required for DC specification, since constitutive MafB expression inhibited DC differentiation. Consistent with this, PU.1 directly bound to MafB, inhibited its transcriptional activity in macrophages, and repressed its ability to induce macrophage differentiation in chicken myeloid progenitors. We propose that high PU.1 activity favors DCs at the expense of macrophage fate by inhibiting expression and activity of the macrophage factor MafB. (Blood. 2005;105: 2707-2716)

Introduction

Dendritic cells (DCs) serve a crucial function in the immune system as the major antigen presenting cells with the unique ability to activate naive T cells,[1] a capacity that has made them the major target of therapeutic manipulation in vaccination and cancer immunotherapy protocols.[1] They can develop from myeloid progenitors in the bone marrow or circulating blood monocytes.[2] When exiting the blood stream, monocytes can give rise to inflammatory macrophages or to antigen presenting DCs.[3] A disturbance of this balance in favor of DC differentiation has been observed in autoimmune disease,[4] whereas tumors can skew it toward the macrophage option.[5]

Cytokine conditions favoring outgrowth of DCs in vitro have been well defined in mouse and human cells, most commonly culture of bone marrow progenitors with granulocyte-macrophage colony-stimulating factor (GM-CSF), $CD34^+$ progenitors with GM-CSF and tumor necrosis factor α (TNFα), or stimulation of blood monocytes with GM-CSF and interleukin-4 (IL-4).[2] By contrast M-CSF,[5] IL-6,[5,6] IL-10,[7] and interferon-γ (IFN-γ)[8] favor macrophage differentiation at the expense of DC fate.

In contrast to the cytokine requirements, the transcriptional events controlling the choice of macrophage versus DC fate have remained unresolved. Some transcription factor knockout mice show defects in DC differentiation[9,10] but also in other lineages, making it difficult to determine at what stage the differentiation block occurred. Certain transcription factor oncogenes have been shown to be capable of transforming DCs or their progenitors, but how this relates to the normal differentiation program remains unclear.[11,12] Constitutive overexpression of myeloid transcription factors in heterogeneous populations of hematopoietic progenitors also has defined factors incompatible with alternative Langerhans cell or granulocyte fates under GM-CSF differentiation conditions.[13] However, the transcription factors that instruct the commitment of myeloid-restricted progenitors or monocytes to DC versus macrophage fate are unknown.

To address this question we have used a hormone-inducible transcription factor and 2 different cell-culture systems that make it possible to analyze uniform clonal-cell populations expressing a transduced gene. In one of these, primary transformed avian myeloblasts can differentiate into granulocytes or macrophages under appropriate conditions.[14] We have previously shown that the expression of the monocyte/macrophage-specific bZip transcription factor MafB in this system favored macrophage differentiation, whereas expression of the myeloid- and lymphoid-specific Ets family factor PU.1 did not.[14]

Gene inactivation studies in mice have revealed that PU.1 is required for the correct development of both the lymphoid and the

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Thomas Graf Albert Einstein College of Medicine, United States of America IMMUNOLOGY New Finding	**What determines whether a myeloid precursor acquires a macrophage or a dendritic cell fate? This paper suggests that it is the balance between the transcription factors PU.1 (Ets family) and MafB (bZip family).** Thus, the ratio of MafB to PU.1 is higher in macrophages than in dendritic cells and enforced expression of PU.1 in monocytes favors dendritic differentiation, whereas MafB skewes them towards a macrophage fate. This appears to occur through a direct antagonism: PU.1 inhibits the transcriptional activity of MafB by directly interacting with the protein. Evaluated 2 Feb 2005

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Cell

MafB Restricts M-CSF-Dependent Myeloid Commitment Divisions of Hematopoietic Stem Cells

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SUMMARY

While hematopoietic stem cell (HSC) self-renewal is well studied, it remains unknown whether distinct control mechanisms enable HSC divisions that generate progeny cells with specific lineage bias. Here, we report that the monocytic transcription factor MafB specifically restricts the ability of M-CSF to instruct myeloid commitment divisions in HSCs. MafB deficiency specifically enhanced sensitivity to M-CSF and caused activation of the myeloid master-regulator PU.1 in HSCs in vivo. Single-cell analysis revealed that reduced MafB levels enabled M-CSF to instruct divisions producing asymmetric daughter pairs with one PU.1^{+} cell. As a consequence, *MafB*$^{-/-}$ HSCs showed a PU.1 and M-CSF receptor-dependent competitive repopulation advantage specifically in the myelomonocytic, but not T lymphoid or erythroid, compartment. Lineage-biased repopulation advantage was progressive, maintained long term, and serially transplantable. Together, this indicates that an integrated transcription factor/cytokine circuit can control the rate of specific HSC commitment divisions without compromising other lineages or self-renewal.

INTRODUCTION

The different short-lived cell types of mammalian blood are continuously regenerated from a small population of hematopoietic stem cells (HSCs) in the bone marrow (Bryder et al., 2006). Although a significant proportion of HSCs with long-term reconstitution potential is predominantly quiescent or divides infrequently (Wilson et al., 2008), HSCs need to enter the cycle to continuously regenerate mature blood cells in a correctly balanced ratio or to replenish the stem cell pool under stress conditions. Cell division of HSCs may thus result in self-renewal divisions or the production of more differentiated progeny (Orford and Scadden, 2008). Although such downstream progenitors still retain a high degree of multipotency, recent advances in their characterization also suggest that early diversification into cells with distinct lineage bias can occur at the most primitive stem and precursor cell level (Dykstra et al., 2007; Iwasaki and Akashi, 2007; Luc et al., 2008). However, the mechanisms controlling such specific lineage engagement divisions remain elusive.

Several cellular regulators have been identified that can either promote or restrict HSC cycling, but their mutation in genetic models exclusively affected self-renewal (Orford and Scadden, 2008; Zon, 2008). By contrast, regulators that selectively control lineage-specific commitment divisions of HSCs have not been identified. In this context, the importance of both transcription factor and cytokine signaling for lineage engagement has been invoked (Metcalf, 2007, 2008; Orkin and Zon, 2008; Sieweke and Graf, 1998; Zhang and Lodish, 2008), but no clear mechanism has emerged as to how these two critical control elements might be integrated. Transcription factors with effects on stem cell cycling so far were exclusively found to affect self-renewal divisions (Orford and Scadden, 2008; Zon, 2008). As for cytokine receptors, several of them are expressed on primitive hematopoietic stem and precursor cells (Akashi et al., 2003; Hu et al., 1997; Miyamoto et al., 2002), but it has been a long-standing debate whether cytokine signaling has instructive or permissive

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MafB restricts M-CSF-dependent myeloid commitment divisions of hematopoietic stem cells.

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Faculty Comments & Author Responses

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New Finding

Comments

This is one of two recent studies that, for the first time, conclusively show that cytokines can have an instructive rather than a permissive role in the differentiation of hematopoietic stem cells (in this paper) or committed myeloid progenitor cells {1}.

Whether cytokines act in an instructive (i.e. drive differentiation of a progenitor or stem cell into a particular lineage) or permissive (i.e. selectively expand precommitted cells within an intrinsically heterogeneous progenitor or stem cell population) fashion in hematopoietic differentiation has been an extremely contentious area of research in hematopoiesis. In this paper, it is shown that deletion of the transcription factor MafB induces responsiveness of hematopoietic stem cells to the cytokine macrophage colony-stimulating factor (M-CSF), leading to preferential differentiation into the myeloid lineage. The experiments are very rigorous. Of note is the fact that the authors present convincing evidence that, after deletion of MafB, M-CSF induces asymmetric cell division of highly purified stem cells into one cell expressing the myeloid transcription factor PU.1 and one cell that is negative for this transcription factor, an experiment that addresses another controversial feature of hematopoietic stem cells, their capacity to divide asymmetrically. Together, the data show that at least M-CSF can play an instructive role in hematopoietic stem cell differentiation. It is interesting to note that another paper was recently published where it was shown that, at the level of committed granulocyte/macrophage progenitor cells, M-CSF instructs differentiation into macrophages, while granulocyte colony-stimulating factor instructs granulocytic differentiation, further supporting an instructive role of at least some cytokines in myeloid differentiation {1}.

References: {1} Rieger et al. Science 2009, 325:217-8 [PMID:19590005].

MafB/c-Maf Deficiency Enables Self-Renewal of Differentiated Functional Macrophages

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In metazoan organisms, terminal differentiation is generally tightly linked to cell cycle exit, whereas the undifferentiated state of pluripotent stem cells is associated with unlimited self-renewal. Here, we report that combined deficiency for the transcription factors MafB and c-Maf enables extended expansion of mature monocytes and macrophages in culture without loss of differentiated phenotype and function. Upon transplantation, the expanded cells are nontumorigenic and contribute to functional macrophage populations in vivo. Small hairpin RNA inactivation indicates that continuous proliferation of MafB/c-Maf deficient macrophages requires concomitant up-regulation of two pluripotent stem cell–inducing factors, KLF4 and c-Myc. Our results indicate that MafB/c-MafB deficiency renders self-renewal compatible with terminal differentiation. It thus appears possible to amplify functional differentiated cells without malignant transformation or stem cell intermediates.

The nonproliferative state of terminally differentiated cells is assured by robust, often redundant mechanisms (*1*, *2*), and in rare exceptions where fully mature cells can re-enter the cycle, proliferation remains transient and/or involves de-differentiation (*3*). It remains unknown what renders differentiated cells refractory to the same mitogen signals that stimulate the proliferation of their direct precursors. For example, the proliferative response of myelomonocytic progenitors to macrophage colony-stimulating factor (M-CSF) is lost upon differentiation to macrophages (*4*), despite the continued ability of these mature cells to sense the cytokine (*5*). Consequently, myeloid progenitor cells form colonies in M-CSF containing semisolid medium, whereas blood monocytes and tissue macrophages do not. Here, we have investigated whether this process involves the transcription factors MafB and c-Maf, which can both regulate M-CSF responsiveness (*6*, *7*) and stimulate monocytic differentiation (*8*–*10*).

Intriguingly, we observed that in contrast to wild-type (WT) cells, MafB/c-Maf double deficient (Maf-DKO) blood leukocytes formed colonies in M-CSF containing medium at high efficiency (Fig. 1A). Several lines of evidence indicated that these colonies were initiated by mature monocytes rather than by other mature cell types or circulating progenitors. First, in a cytokine mix that can reveal rare circulating stem and progenitor cells (*11*), Maf-DKO leukocytes gave rise to the same number of colonies as WT cells, an amount that was lower by a factor of 100 than in M-CSF (fig. S1A). Furthermore, M-CSF colonies did not develop from lymphocytes or granulocytes (fig.S1C) but formed at very high frequency from purified mature Maf-DKO monocytes that expressed Mac-1, F4/80, and CD115 but were negative for c-kit (CD117), a marker of both primitive (*11*) and M-CSF–responsive macrophage/dendritic cell progenitors (*12*) (Fig. 1B and fig. S2). A high rate of colony formation was also observed for spleen and peritoneal macrophages (fig. S1B) as well as for purified $CD117^-$ Kupffer cells of the liver, which represent terminally differentiated tissue macrophages (Fig. 1B). These results indicated that, in contrast to WT, mature Maf-DKO blood monocytes and tissue macrophages could proliferate in response to M-CSF.

Indeed, when we injected recombinant M-CSF directly into the circulation of mice, bromodeoxyuridine (BrdU) incorporation and 7-aminoactinomycin staining revealed that Maf-DKO, but not WT, blood monocytes had entered the cell cycle (Fig. 1C). The same observation was made when cells were stimulated with M-CSF ex vivo (fig. S1D). Although Maf-DKO monocytes or in vitro differentiated macrophages required M-CSF concentrations for proliferation that were at least 10 times as high as bone marrow progenitors (fig. S3), their ability to divide was not restricted to a small number of cycles but continued in extended long-term culture. Maf-DKO monocyte–derived colonies could thus be serially replated in methocult assays at high efficiency and without loss of clonogenicity (Fig. 1D). This was intriguing, because even progenitors normally have only limited replating ability in this assay that is often employed to reveal the extended self-renewal capacity of transformed progenitors (*13*). Moreover, extended, possibly unlimited, expansion of Maf-DKO cells could be achieved in liquid culture. We have maintained Maf-DKO cells in continuous culture for more than 8 months without any signs of crisis. For three independent Maf-DKO populations, cell counts over 2 months revealed stable doubling times of 1.44 ± 0.05 days and theoretical amplification factors of 10^{11} to 10^{12} (fig. S4A). This was unlikely to be due to the outgrowth or selection of a small subpopulation; more than 80% of Maf-DKO cells gave rise to new colonies in replating assays (Fig. 1D), and individual colonies could be cloned and subcloned at 80 to 90% efficiency (fig. S4B) or undergo expansion in liquid culture with similar, unaltered growth curves (Fig. 1E). Together, the high cloning and recloning efficiency and the similar growth rates of individual clones indicates that a vast majority, possibly all, rather than a subpopulation of Maf-DKO cultures have an extended proliferation capacity.

In specialized regenerative processes, differentiated cells can also reenter the cell cycle, but in these examples cells typically undergo dedifferentiation and revert to an immature phenotype (*3*). By contrast, proliferating Maf-DKO cells maintained a differentiated phenotype and function. Like WT monocytes in M-CSF culture, Maf-DKO cells remained positive for the monocyte/macrophage surface markers FcγRII/III (CD16/32), Mac-1 (CD11b), F4/80, and CD115 (Fig. 2A); displayed a normal macrophage morphology (Fig. 2B); and were negative for progenitor markers CD117 and CD34 (Fig. 2A) or other lineage markers (fig. S5A), even after long-term expansion. Proliferating Maf-DKO cells also displayed a global gene expression profile highly similar to WT cells (Fig. 2C) and expressed a panel of characteristic monocyte/macrophage genes (Fig. 2D and fig. S5B). They also showed the same capacity as WT monocyte–derived macrophages to produce nitric oxide in response to lipopolysaccharide and interferon-γ (Fig. 2E) and to phagocytose latex beads (Fig. 2F). Cell cycle analysis further demonstrated that Maf-DKO cells in S and G2/M phase had the same amount of phagocytic activity as cells in G0/G1 phase, indicating that differentiated macrophage function is fully maintained through cell cycle progression in these cells (Fig. 2G). This was further confirmed with live bacteria, revealing that cycling ($Ki67^+$) Maf-DKO cells could phagocytose large numbers of green fluorescent protein (GFP)–expressing *Salmonella typhimurium* (Fig. 2H). Interestingly, M-CSF–expanded monocyte-derived Maf-DKO cells retained the ability to acquire dendritic cell features when shifted to GM-CSF–containing medium (fig. S6A and B).

To determine whether the extended proliferative capacity of Maf-DKO monocytes and macrophages was associated with tumorigenic transformation, we analyzed the long-term effects of MafB/cMaf deficiency in vivo. Interest-

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