Josef Bodor, Ph.D.

Work: **Institute of Experimental Medicine AS CR, v.v.i., EU Centre of Excellence**
Videnska 1083
142 20 Prague 4-Krc
Czech Republic
Europe
Telephone: +420 241 062 570
Fax: +420 241 062 785

Privat: Jablonecka 7/716
190 00 Prague 9
Czech Republic
Telephone: +420 286 580 456
iPhone: + 420 722 950 013
E-mail: josef.bodor@biomed.cas.cz

I. Studies and Appointments

Education:

INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
Institute of Molecular Genetics, Prague, (CR)	Ph.D.	1990	Mol.Oncology
University of J.E. Purkynje, Brno (Czech Rep)	M.Sc.	1984	Mol. Biology

Faculty Appointments:

-2011 – 2012 **Visiting Professor,** Institut für Immunologie, Universitätmedizin JGU Mainz, Germany.

-2008 – 2011 **Visiting Professor,** University of Würzburg, Department of Molecular Pathology, Würzburg, Germany. **Associate Member of Transregio 52** entitled 'Transcriptional Programming of Individual T cell subsets'.

-2005 - 2007 **Assistant Professor**, Columbia University, College of Physicians and Surgeons, Department of Pathology, New York, NY.

-2004 – 2005 **Visiting Professor**, Kyoto University, Institute of Frontier Medical Sciences, Department of Experimental Pathology, Kyoto, Japan.

- 2001- 2003 **Assistant Professor of Medicine**, Boston University, School of Medicine, Boston, MA.

- 1995 -1997 **Instructor in Medicine at Harvard Medical School**, Boston, MA.

Appointments:

2013 - **Senior Investigator,** Institute of Experimental Medicine AS CR, v.v.i. EU Center of Excellence.

-2011 – 2012 **Visiting Professor,** Institut für Immunologie, Universitätmedizin JGU Mainz, Germany.

-2008 – 2011 **Visiting Professor,** University of Würzburg, Department of Molecular Pathology, Würzburg, Germany.

-2007- 2008 **Senior Scientist,** Institute of Microbiology, Academy of Czech Republic, Prague, Czech Republic

-2005- 2007 **Assistant Professor**, Columbia University, College of Physicians and Surgeons, Department of Pathology, New York, NY.

-2004 – 2005 **Visiting Professor**, Kyoto University, Department of Experimental Pathology, Kyoto, Japan. Faculty member investigating role of potent transcription repressors ICER (inducible cAMP early repressor) and

CREM (cAMP response element modulator) in suppressive function of regulatory T cells.

- 2001-2003 **Assistant Professor of Medicine**, Boston University, School of Medicine, Department of Medicine. Performed research of immunoregulatory function of ICER in lymphocytes and its role in HIV-mediated dysfunction.

- 2000-2001 **Director of Virology**, BioReliance, Rockville, MD. Head of Virology Department consisting of Retrovirology and General Virology laboratories. Study Director for GLP and cGMP assays designed to detect retroviruses, adenoviruses, bovine, and porcine viruses in biologics intended for human therapy.

- 1997-1999 **Senior Biotechnology Fellow**, National Cancer Institute, Experimental Immunology Branch, Bethesda, MD, supervisor: Dr. Ronald E. Gress in the Experimental Immunology Branch headed by Dr. Al Singer. Expanded study of the role of ICER in regulation of gene transcription in immunocytes. Discovered that ICER represses FasL and MIP-1β in addition to IL-2 transcription. Studied differential gene expression in Th1 versus Th2 cells, in NK cells, and monocytes. Published review on role of ICER in induction of anergy in T lymphocytes. Filed patent application with NCI for use of anti-ICER therapy for treatment of cancer and infectious diseases.

- 1993 -1997 **Research Associate**; Howard Hughes Medical Institute, Massachusetts General Hospital, Harvard Medical School, Boston, MA, supervisor: Dr. Joel F. Habener. Discovered that member of CREB/CREM family transcription repressor ICER, represses IL-2, TNF-α, GM-CSF, and IL-4 gene expression in T lymphocytes. Also proved that ICER inhibits expression of HTLV-I.

-1992 - 1993 **Research Fellow**; Division of Human Retrovirology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, supervisor: Dr. William A. Haseltine (Dr. Haseltine left for HGS). Demonstrated that CREB/CREM family of transcription factors takes part in regulation of HTLV-I expression.

-1990 - 1992 **Research Fellow**, Fred Hutchinson Cancer Research Center, Seattle, WA, supervisor: Dr. Maxine Linial. Designed a screening method to select temperature sensitive mutants of HIV-1 Tat protein. This method utilized MLV-based retroviral vectors, mutagenesis and flow cytometry based phenotypical sorting.

-1984 - 1990 **Graduate Student**, Institute of Molecular Genetics, Czech Academy of Sciences, Prague, Czech Republic, supervisor: Dr. Jan Svoboda. Molecularly cloned and sequenced unique provirus of Rous sarcoma virus (RSV). Proved that this deletion mutant emerged by alternative splicing and reverse transcription of v-*src* mRNA.

-1979 - 1984 **Research Assistant**, Institute of Biophysics, Czech Academy of Sciences, Brno, Czech Republic, supervisor: Dr. Emil Palecek. Isolated and characterized DNA-topoisomerase I from chicken erythrocytes.

II. Honors/Awards:

-2011 *Ad hoc* reviewer, Arthritis & Rheumatism, Expert Opinion On Therapeutic Targets, Experimental Cell Research.

-2010 *Invited lecture*, World Immune Regulation Meeting, IV, Davos, March 29 – April 1st, 2010, Switzerland.

-2009 *Invited lecture*, 2nd European Congress of Immunology, Berlin, September 13-16, 2009, Germany.

-2009 *Invited lecture*, 12th German Meeting on T cells: Subsets and Functions University of Marburg, Marburg, Juni 24-25, 2009, Germany.

-2008 *Invited lecture*, Transregio 52, Transcriptional Programming of Individual T Cell Subsets, Johannes Gutenberg University, Mainz, Germany
-2008 *Invited lecture*, Pathology Seminar Series, Institute of Pathology, University of Wuerzburg, Germany
-2006 *Invited lecture*, Pathology Seminar Series, Institute of Pathology, University of Wuerzburg, Germany
-2006 *Invited lecture*, Department of Virology, University of Ulm, Germany
-2006 *Invited lecture*, Institute of Human Virology Annual Meeting, Baltimore, MD
-2006 *Invited lecture*, Columbia University, Annual Retreat of Dept of Microbiology and Immunology, IBM Center, NJ.
-2006 *Invited lecture*, The 6th International Cytokine Conference 2006, Vienna, Austria
-2006 *Third Prize, Young Investigator*, The 6th International Cytokine Conference 2006, Vienna, Austria
-2005 *Invited lecture*, Joint-DFCI-BWH-JDC Immunological Seminar Series, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA.
-2005 Invited lecture, Neuroimmunology Seminar Series, Brigham Women's Hospital, Harvard Medical School, Boston, MA.
-2004 *Invited lecture*, Institute of Frontier Medical Sciences, Kyoto University, Japan
-2003 *Invited lecture*, The Queens University of Belfast, Belfast, United Kingdom
-2003 *Invited lecture*, Frontiers of Science, Spring Seminars Biocity, Turku, Finland
-2002 *Ad hoc* reviewer, Wellcome Trust, London UK
-1999 *Invited lecture*, HIV, Leukemia, and Opportunistic Cancers, Meeting of Harvard AIDS Institute, Marrakech, Morroco.
-1999 *Invited lecture*, International Meeting of the Institute of Human Virology, Baltimore, MD
-1998 FARE Award, National Institutes of Health
-1996 *Invited lecture*, ICE'96 Xth International Congress on Endocrinology, San Francisco, CA
-1995 *Invited lecture*, 77th Annual Meeting of The Endocrine Society, Washington, D.C.
-1995 Corning Nichols Young Investigator Award
-1991 NIH Grant RO3
-1990 *Invited lecture*, Universite de Luminy, Marseilles, France
-1990 Ph.D. Degree graded "Magnae cum laude" (Thesis title: " Molecular cloning and sequencing of the LTR, v-src, LTR proviral structure H19 and its utilization for preparation of retroviral vector" Prague, Czech Republic)
-1989 *Invited lecture*, Institut Pasteur, Paris, France
-1989 *Invited lecture*, Institut Jacques Monod, Paris, France
-1989 Federal Research Award at Institut Pasteur, Paris, Federal Student Association
-1985 Federal Research Award Czechoslovakia, Federal Student Association

III. Patents/Inventions:

PCT/US International Patent application (Publication number WO 99/43814) filed August 22nd, 2000): "Use of Antisense Therapy to Block Synthesis of the Transcriptional Repressor ICER to Render the Immune System Nonsuppressible by Cancer and Infectious Pathogens". Inventors: P.A. Cohen, **J. Bodor**, D.E. Weng, G.K. Koski, B.J. Czerniecki, J. Bodorova.

IV. Areas of Specialization

Differential gene expression, protein purification, transcription and cell signal transduction, knowledge of retroviral and HIV field, molecular immunology, transcriptional regulation of cytokine and chemokine genes in T cells, NK cells, monocytes, and dendritic cells; regulation of chemokine and chemokine receptor expression, retroviral vector development technology; manipulation with large DNA fragments; gene delivery; design and development of gene expression systems, working knowledge of GLP and cGMP

V. Areas of Expertise

Protein Purification: Isolation of eukaryotic Topoisomerase I from chicken erythrocytes on hydroxylapatite column, isolation of several restriction enzymes (Xba I, Sph I, Xho I) on ion exchange columns. Expression and purification of recombinant proteins of ICER and CREB in bacteria as a glutathione S-transferase (GST) fusion proteins. Expression and purification of NFATp in bacteria as a hexahistidine-tagged protein. Transient overexpression of various proteins in Jurkat, and Cos cells under various eukaryotic promoters.

Gene Therapy Vectors: HIV- and MLV-based vectors; Rous sarcoma virus (amplicon vectors, recombinant viruses); retroviral hybrid vectors, virus production and analysis, Projects involving lentiviral vectors and RNAi strategies.

Molecular biology: Cloning (genomic library construction,cDNA isolation, gene screening, subcloning). Design and construction of mammalian expression vectors

Gene expression (Microarray technology, Differential Display, Northern blot, RNase protection, primer extension, RT-PCR, real time PCR, *in-situ* hybridization, gel shift assay, transcription reporter assays using CAT and luciferase reporters). Gene analysis and screening (Southern blot, DNA sequencing,)

Eukaryotic cell culture: Mammalian cell culture (human PBL primary cells- cortical and medullary thymocytes,differentiation of Th1 and Th2 type T cells monocytes into dendritic cells; analysis of subsets of primary T, NK cells, B cells, monocytes, and dendritic cells). MoFlow sorting of mouse $CD25^+$ regulatory T cells, regulatory T cell assays, thymidine incorporation proliferation assays. Transfection and expression of foreign genes, episomal eukaryotic vectors, clonal analysis, antisense oligonucleotides treatment.

Biochemistry: Enzymatic assays, ELISA, Western blot, immunoprecipitation immunocytochemistry, immunohistochemistry, NEPHGE, protein expression, isolation and purification of proteins in bacteria and mammalian tissue

Chemistry: Analytical methods (affinity chromatography, gel filtration)

VI. Publications:

Reviews and Chapters in Books

1. **Bodor, J.** 1985. Topoisomerases. Biologicke Listy (Abstract in English), 50: 123-140.

2. Svoboda, J., Geryk, J., Pichrtova, J., Dvorak, M., Karakoz, I., Nehyba, J., **Bodor, J.,** Guntaka, R., Kandala, J. 1989. Rescue, transmission and characterization of cryptic LTR, v-src, LTR provirus. *Highlights of Modern Biochemistry* (ed. A. Kotyk, J. Skoda, V. Paces, and V. Kostka), pp. 615-627, VPS International Science Publishers, Zeist.

3. **Bodor, J**., Bodorova, J., Gress, R. E. 2000. Suppression of T cell function: A potential role for transcriptional repressor ICER. (Review) *Journal of Leukocyte Biology, 67: 774-779.*

4. **Bodor, J.,***Fehervari, Z. Diamond, B., Sakaguchi, S., 2007. Regulatory T cell mediated suppression: potential role of ICER. Review. *Journal of Leukocyte Biology, 81 161-167.*

5. Vaeth, M., Gogishvili, T., Bopp, T., Berberich-Siebelt, F., Klein-Hessling, S., Schmitt, E., Huenig, T., Serfling, E., **Bodor, J.*** 2009. Cyclic AMP-mediated shuffling of ICER alters

NFAT-dependent suppression by regulatory T cells. 2nd European Congress of Immunology (ed. R.E. Schmidt), pp. 137-143, Medimond International Proceedings, Bologna, Italy.

6. **Bodor, J*.**, Bopp, T., Vaeth, M., Klein, M., Serfling, E., Hünig, T., Becker, C., Schild, H., and Schmitt, E. Cyclic AMP underpins suppression by regulatory T cells. (Review) *European Journal of Immunology 42: 1375-1384, 2012.*

Original Reports

7. **Bodor, J**., and Svoboda, J. 1989. The LTR, v-src, LTR provirus generated in the mammalian genome by src mRNA reverse transcription and integration. *J. Virol. 63: 1015-1018.*

8. **Bodor, J**., Poliak, E., Pichrtova, J., Geryk, J., Svoboda, J. 1989. Complete nucleotide sequence of LTR, v-src, LTR provirus H-19. *Nucl. Acid. Res. 17:8869.*

9. Svoboda, J., Geryk, J., **Bodor, J.,** Pichrtova, J., Plachy, J. Genesis and oncogenic activity of the LTR, v-src, LTR provirus.1990. Supplement to *J. Canc. Res. and Clin. Oncol. Springer* Vol. 116, pp. 1060, 15th International Cancer Congress, Hamburg, August 16-22.

10. **Bodor, J**., Habener, J. 1995. Modulation of HTLV-I promoter expression by Tax and PKA via cAMP response element (CRE) binding protein CREB and CRE-modulator protein CREM. Supplement (19A) to *J. of Cell. Biochem*, pp. 30, Keystone, CO, USA, January 5-26.

11. **Bodor, J**., Walker, W., Flemington, E., Spetz, A.-L., Habener, J. F. 1995. Modulation of Tax and PKA-mediated expression of HTLV-I promoter via cAMP response element binding and modulator proteins CREB and CREM. *FEBS Letters 377: 413-418.*

12. **Bodor, J**., Spetz, A.-L., Strominger, J. L., Habener, J. F. 1996. Cyclic AMP inducibility of transcriptional repressor ICER in developing and mature human T lymphocytes. *Proc. Natl. Acad. Sci. USA 93: 3536-3541.*

13. **Bodor, J***., Habener, J.F. 1998. Role of transcriptional repressor ICER in cyclic AMP-mediated attenuation of cytokine gene expression in human thymocytes. *J. Biol. Chem. 273: 9544-9551.*

14. **Bodor, J*.**, Bodorova, J., Feigenbaum, L., Barabitskaja, O., Reitz, M., Gallo, R.C., Gress, R.E. 1999. ICER represses MIP-1α and MIP-1β expression in stimulated subsets of human T cells; possible role of ICER in cAMP-mediated downregulation of CCR5 expression in monocyte-derived macrophages. *Journal of Human Virology 2: 198.*

15. Kirshner, S., Palmer, L., **Bodor, J.**, Saji, M., Kohn, L.D., Singer, D.S. 2000. Major histocompatibility class I gene transcription in thyrocytes: a series of interacting regulatory DNA sequence elements mediate thyrotropin/cyclic adenosine 3', 5'-monophosphate repression. *Molecular Endocrinology. 14: 82-98.*

16. **Bodor, J.,** Feigenbaum, L., Bodorova, J., Bare, C., Reitz, M.S., Gress, R.E. 2001. Suppression of T-cell responsiveness by inducible cAMP early repressor (ICER). *Journal of Leukocyte Biology, 69; 1053-1069.*

17. **Bodor, J.**, Bodorova, J., Bare, C., Hodge, D., Young, H.A., Gress, R.E. 2002. Differential inducibility of the transcriptional repressor ICER and its role in modulation of Fas ligand expression in T and NK lymphocytes. *European Journal of Immunology, 32: 203-212.*

18. Barabitskaja, O., Foulke, J.S., Pati, S., **Bodor, J.,** Reitz, M.S. 2006. Suppression of MIP-1β transcription by inducible cAMP early repressor (ICER). *Journal of Leukocyte Biology, 79: 378-387.*

19. **Bodor, J.*,** 2006 Regulation of HIV-1 and IL-2 transcription by inducible cAMP early repressor (ICER). Retrovirology, 3: (Supplement I: S81).

20. **Bodor, J.,*** Fehervari, Z., Diamond, B., Sakaguchi, S., 2007. ICER/CREM – mediated transcriptional attenuation of IL–2 and its role in suppression by regulatory T cells. *European Journal of Immunology, 37: 884-895.*

21. Vaeth, M., Gogishvili, T., Bopp, T., Klein, M., Gattenloehner, S., Berberich-Siebelt, F., Avots, A., Sparwasser, T., Grebe, N., Schmitt, E., Hünig, T., Serfling, E., and **Bodor, J.*** 2011. Regulatory T cells facilitate the nuclear accumulation of inducible cAMP early repressor (ICER) and suppress nuclear factor of activated T cell c1 (NFATc1). *Proc. Natl. Acad. Sci. USA, 108: 2480-2485*.

22. **Bodor, J*.,** Bopp, T., Vaeth, M., Klein, M., Serfling, E., Hünig, T., Becker, C., Schild, H., and Schmitt, E. *Cyclic AMP underpins suppression by regulatory T cells. European Journal of Immunology 42: 1375-1384, 2012.*

23. Klein, M., Ullrich, N., Klein-Hessling, S., Becker, M., **Bodor, J.** Serfling, E., Bopp, T., Schmitt, E. The role of IKAROS transcription factor HELIOS in nTreg development and function. *In preparation.*

*** Corresponding Author**

Invited Presentations:

1989 Institut Pasteur, Paris, France.
1989 Institut Jacques Monod, Paris, France.
1990 Universite de Luminy, Marseilles, France.
1991 NIAID/NIH, Washington, D.C.
1991 University of California, Los Angeles, CA.
1995 77th Annual Meeting of The Endocrine Society, Washington, D.C.
1996 ICE'96 Xth International Congress on Endocrinology, San Francisco, CA.
1996 John Hopkins University, Baltimore, MD.
1996 Institute of Human Virology, Baltimore, MD.
1999 HIV, Leukemia, and Opportunistic Cancers, Meeting of Harvard AIDS Institute,Marrakech, Morroco.
1999 International Meeting of the Institute of Human Virology, Baltimore, MD.
2003 Boston University, Boston, MA.
2003 Frontiers of Science, Spring Seminars, Biocity, Turku, Finland
2003 The Queens University of Belfast, Belfast, United Kingdom
2004 Frontier Institute of Medical Sciences, University of Kyoto, Kyoto, Japan.
2005 Neuroimmunology Seminar Series, Brigham Women's Hospital, Harvard Medical School, Boston, MA.
2005 Joint-DFCI-BWH-JDC Immunological Seminar Series, Harvard Medical School, Boston, MA.
2006 The 6th International Cytokine Conference 2006, Vienna, Austria.
2006 Columbia University, Annual Retreat of Dept of Microbiology and Immunology, IBM Center, NJ.
2006 Institute of Human Virology Annual Meeting, Baltimore, MD.
2006 Pathology Seminar Series, Institute of Pathology, University of Wuerzburg, Germany.
2006 University of Ulm, Ulm, Germany.
2008 Transregio 52, Transcriptional Programming of Individual T Cell Subsets, Johannes Gutenberg University, Mainz, Germany.
2009 The 12th German Meeting on T cells: Subsets and Functions University of Marburg, Marburg, Juni 24-25, 2009, Germany.
2009 2nd European Congress of Immunology, Berlin, September 13-16, 2009, Germany.
2010 World Immune Regulation Meeting, IV, Davos, March 29 – April 1st, 2010, Switzerland.

VII. Other Skills

1. Supervising Laboratory Managers, Members of Technical Staff, and Students
2. Language skills: fluent in English, Russian, native Czech, and Slovak

3. Computer skills

Abstracts and Posters at Meetings:

1. **Bodor, J.** Characterization of the LTR, v-src, LTR structure integrated in the mammalian genome. Oral presentation, September 7th 1989, Institut Jacques Monod, Paris, and September 8th 1989, Institut Pasteur, Paris, France.

2. Svoboda, J., Geryk, J., Pichrtova, J. Nehyba, J., **Bodor, J**., Guntaka, R., Kandala, J., Dezelee, P., Calothy, G. Generation of sarcomas by reverse transcription of v-src mRNA and by novel transduction of c-src. Abstracts of paper presented at the XVIII Meeting of the Tumor Virus Group April 30 - May 4, 1989, Sundbyholm's Castle, Sweden, pp. 1.

3. **Bodor, J**., Walker, W. H., Fleminton, E., Spetz, A.- L., Habener, J. F. Modulation of HTLV-I LTR promoter expression by Tax and PKA via cAMP response element (CRE) - binding protein CREB and CRE modulator protein CREM. Abstract of paper presented at the Cold Spring Harbor Meeting on Retroviruses, May 24-29, 1994, Cold Spring Harbor, N. Y. USA, pp. 93.

4. **Bodor, J**., Walker, W. H., Habener, J. F. Modulation of HTLV-I promoter expression by Tax and PKA via cAMP response element CREB and CRE-modulator protein CREM. Abstract of paper presented at the 76th Annual Meeting of The Endocrine Society, June 15-18, 1994, Anaheim, CA, USA, pp. 571.

5. **Bodor, J**., Spetz, A.-L., Strominger, J. L., Habener, J. F. Cyclic AMP inducibility of transcriptional repressor ICER in mature human T lymphocytes. Abstract of paper presented at the Cold Spring Harbor Meeting on Retroviruses, May 23-28, 1995, Cold Spring Harbor, N. Y. USA.

6. **Bodor, J**., Spetz, A.-L., Strominger, J. L., Habener, J. F. Cyclic AMP inducibility of transcriptional repressor ICER in mature human T lymphocytes. Abstract of paper presented at the 77th Annual Meeting of The Endocrine Society, June 14-17, 1995, Washington, D.C. USA, pp. 66.

7. **Bodor, J.**, Spetz, A.-L., Strominger, J.L., Habener, J.F. Cyclic AMP inducible transcriptional repressor ICER modulates HTLV-I and IL-2 expression in T cells. Oral presentation at the Xth International Congress of Endocrinology, June 12-15, 1996, San Francisco, CA USA, Vol.I, pp 129.

8. **Bodor, J.**, Bodorova, J., Bare, C.V., Gress, R.E. Differential inducibility of the transcriptional repressor ICER and its role in modulation of Fas ligand expression in T and NK lymphocytes. Abstract of paper presented at NIH Immunology Retreat, October 26-28, 1998, Airlie House, Warrenton, Virginia, USA.

9. **Bodor, J.**, Bodorova, J., Gress, R.E. Role of ICER in differential expression of MIP-1α and MIP-1β in stimulated subsets of human T cells with T helper 1 or T helper 2 dominant phenotype. Abstract of paper presented at the Keystone meeting on Chemokines and Chemokine Receptors, January 18-23, 1999 Colorado, USA, pp. 49.

10. **Bodor, J.**, Bodorova, J., Feigenbaum, L., Barabitskaja, O., Reitz, M., Gallo, R.C., Gress, R.E. ICER represses MIP-1α and MIP-1β expression in stimulated subsets of human T cells; possible role of ICER in cAMP-mediated downregulation of CCR5 expression in monocyte-derived macrophages. Oral presentation at International Symposium on HIV, Leukemia, and Opportunistic Cancers, May 23-28, 1999, Harvard AIDS Institute, Marrakech, Morocco, pp 53.

11. **Bodor, J.**, Bodorova, J., Feigenbaum, L., Barabitskaja, O., Reitz, M., Gallo, R.C., Gress, R.E. ICER represses MIP-1α and MIP-1β expression in stimulated subsets of human T cells; possible role of ICER in cAMP-mediated downregulation of CCR5 expression in monocyte-

derived macrophages. 1999 Oral presentation at International Meeting of the Institute of Human Virology, August 28- September 2, 1999, A Symposium on HIV-AIDS & Cancer Biology, Baltimore, MD.

12. **Bodor, J**., Bodorova, J., Bare, C., Hodge, D.L., Young, H.A., Gress, R.E. Differential inducibility of the transcriptional repressor ICER and its role in modulation of Fas ligand expression in T and NK lymphocytes. Gene Expression and Signaling in the Immune System, April 24-28, 2002, Cold Spring Harbor Laboratory, N.Y., USA.

13. Barabitskaja, O., Foulkes, J.S., Pati, S., **Bodor, J.**, Reitz, M.S. Suppression of human MIP-1β transcription by inducible cAMP early repressor (ICER). 2002 International Meeting of the Institute of Human Virology, September 9-13, 2002, Baltimore, MD, USA.

15. **Bodor, J**., Bodorova, J., Bare, C., Hodge, D.L., Young, H.A., Gress, R.E. Differential inducibility of the transcriptional repressor ICER and its role in modulation of Fas ligand expression in T and NK lymphocytes. Basic Aspects of Tumor Immunology, February 17-23, 2003, Keystone, Colorado, CO, USA.

16. **Bodor, J.,** Fehervari, Z., Sakaguchi, S. Inducible cAMP early repressor (ICER) and its role in the inhibitory funcion of regulatory T cells. The 34th Annual Meeting of the Japanese Society for Immunology, December 1-3, 2004, Sapporo, Hokkaido, Japan.

17. **Bodor, J.,** Fehervari, Z., and Sakaguchi, S.ICER/CREM – mediated transcriptional attenuation of IL–2 and its role in suppression by regulatory T cells. Lymphocyte Activation and Signaling. January 6-11, 2006, Steamboat, Colorado, CO, USA.

18. **Bodor, J**., Regulation of HIV-1 and IL-2 transcription by inducible cAMP early repressor (ICER). Institute of Human Virology 10th Annual Meeting, November 17-21, 2006, Baltimore, MD, USA.

19. **Bodor, J.,**.ICER/CREM – mediated transcriptional attenuation of IL–2 and its role in suppression by regulatory T cells. Pathologisches Institut der Universität Würzburg December 20, 2006, Würzburg, Germany.

20. **Bodor, J.,**.ICER/CREM – mediated transcriptional attenuation of IL–2 and its role in suppression by regulatory T cells. Department of Virology, Universität Ulm, December 21, 2006.

21. **Bodor, J.,** Fehervari, Z., Diamond, B., and Sakaguchi, S. Transcriptional attenuation of IL–2 and the role of ICER/CREM in suppression by regulatory T cells. Salk Institute, Nature and Foundation IPSEN, Symposium on Biological Complexity: Diseases of transcription, San Diego, January 11-14, 2007.

22. Vaeth, M., Gogishvili, T., Bopp, T., Berberich-Siebelt, F., Klein-Hessling, S., Schmitt, E., Huenig, T., Serfling, E., **Bodor, J.** Cyclic AMP-mediated shuffling of ICER alters NFAT-dependent suppression by regulatory T cells. 2nd European Congress of Immunology, Berlin, Germany, September 13-16, 2009.

VII. Summary of Research

A MSc. degree with an emphasis on the structure and chemical properties of macromolecules under supervision of Dr. Emil Palecek at Institute of Biophysics, Czechoslovak Academy of Sciences (Brno, Czech Republic) allowed me to study topoisomerases and their effects on topology of circular plasmid DNAs. I purified topoisomerase I from chicken erythrocytes and characterized its interactions and function (Bodor, J**.** Topoisomerases. 1985. **Biologicke Listy (Abstract in English)**, **50: 123-140**).

As part of my doctoral research under supervision of Dr. Jan Svoboda at the Institute of Molecular Genetics, Czechoslovak Academy of Sciences (Prague, Czech Republic), I have molecularly cloned and sequenced an altered avian provirus of Rous sarcoma virus (RSV) generated by a unique event of reverse transcription and normal retroviral integration of regularly spliced v-src mRNA. The presence of an intact viral splice junction, as well as duplications of the chromosomal sequence GCGGGG flanking the two 2-base-pair-deleted LTRs, are evidence that the H-19 provirus found in a mammalian tumor cell line designated H-19, has arisen by encapsidation of v-src mRNA instead of genomic RNA of RSV (Bodor, J., and Svoboda, J. *The LTR, v-src, LTR provirus generated in the mammalian genome by src mRNA reverse transcription and integration.* **J. Virol. 63**: **1015-1018, 1989,** Bodor, J., Poliak, E, Pichrtova, J., Geryk, J., Svoboda, J., *Complete nucleotide sequence of LTR, v-src, LTR provirus H19.* **Nucl Acid Res 17: 8869, 1989**). Furthermore, the LTR, v-*src*, LTR structure formed after *src* mRNA integration has provided after replacement of v-*src* by the *neo* gene a minimal retroviral vector. Most of the above work has been described in my thesis for a candidate-of-sciences degree (Ph.D.).

During my postdoctoral training in the laboratory of Dr. William A. Haseltine at Dana-Farber Cancer Institute, Harvard Medical School (Boston, MA), I started my work on the transcriptional regulation of another retrovirus - Human T-lymphotropic Virus I (HTLV-I), the etiological agent of adult T cell leukemia (ATL) which is thought to be the agent responsible for HTLV-I associated myelopathy (HAM), also called tropical spastic paraparesis (TSP). Transcriptional expression of the HTLV-I is a subject of complex regulation. The prominent role in this regulation is played by three 21-base pair (bp) repeats containing asymmetric cyclic AMP (cAMP) responsive DNA elements (CREs) located within the U3 region of HTLV-I Long Terminal Repeat (LTR) promoter. Viral transactivator Tax and cAMP-responsive protein kinase-A (PKA) are important regulators of HTLV-I gene expression acting through CRE sites in 21-bp repeats of HTLV-I LTR. Since Dr. Haseltine left Dana-Farber Cancer Institute to start his biotechnology company (Human Genome Sciences, Inc.) I transferred and completed this project in Dr. Joel F. Habener's laboratory funded in part by Howard Hughes Medical Institute at Massachusetts General Hospital (Bodor, J., Walker, W., Flemington, E., Spetz, A.-L., Habener, J. F. *Modulation of Tax and PKA-mediated expression of HTLV-I promoter via cAMP response element binding and modulator proteins CREB and CREM***. FEBS Letters 377:413-418, 1995**).

These initial data opened a new line of investigation performed in collaboration with Anna-Lena Spetz from the laboratory of Dr. Jack L. Strominger at Dana-Farber Cancer Institute, Harvard Medical School (Boston, MA), leading to the analysis of more general aspects of the cAMP/PKA pathway in developing and mature human T lymphocytes. Despite that the antiproliferative effects of cAMP in stimulated T cells have been described a long time ago, little has been known about the mechanism by which they are initiated. When activated T lymphocytes undergo stimulation accompanied by events that elevate intracellular cAMP levels, they become quiescent by a mechanism, which is unclear. The ability of T lymphocytes to form high intracellular levels of cAMP acquired during T cell development in human thymus is retained by the majority of mature peripheral blood T cells. Importantly, elevated cAMP levels correlate with the induction of a potent transcriptional repressor ICER (Inducible cAMP Early Repressor), a member of the CREB/CREM family of transcription factors, previously described only in certain tissues of the hypothalamic-pituitary-gonadal axis. Importantly, in T lymphocytes, ICER inhibits calcineurin-mediated interleukin 2 (IL-2) expression as well as Tax-mediated transactivation of the HTLV-I promoter. ICER is the only inducible transcription factor of CREB/CREM family identified so far, due to its unique internal intronic P2 promoter located downstream of CREM transactivation domain. The lack of transactivation domain inherently defines ICER as a potent transcriptional repressor. Moreover, ICER is the only member of CREM family of transcription factors expressed in immunocytes. Cyclic AMP autoregulatory response elements (CAREs) within P2 promoter, responsible for characteristic cyclical expression of ICER, have capacity to inhibit ICER's own promoter. Thus, cyclical induction of ICER in T cells may play an important role not only in cAMP induced quiescence but also in the persistent latency of HTLV-I (Bodor, J., Spetz, A.-L., Strominger, J. L., Habener, J. F. *Cyclic AMP inducibility of transcriptional repressor ICER in developing and mature human T lymphocytes*. **Proc. Natl. Acad. Sci. USA 93:3535-3541, 1996**;

Bodor, J., Habener, J.F. *Role of transcriptional repressor ICER in cyclic AMP-mediated attenuation of cytokine gene expression in human thymocytes*. **J. Biol. Chem. 273: 9544-9551, 1998).**

The chemokine receptor CCR5 can function as a co-receptor for HIV entry into $CD4^+$ T cells and macrophages, especially during early stages of HIV infection. The regulation of CCR5 receptor expression may affect leukocyte migration, as well as infection by HIV-1 and, therefore, acquired immunodeficiency syndrome (AIDS) pathogenesis. PGE_2, a well know agonist capable to increase intracellular levels of cAMP, downregulates CCR5 expression in parallel with robust induction of potent transcriptional repressor ICER. In monocytes pretreated with PGE_2 this correlates with consequent loss of CCR5 expression and reduced HIV-1 entry into monocyte-derived macrophages as well as reduced intracellular calcium mobilization in response to the cognate CCR5 ligand, MIP-1β. Importantly, downregulation of CCR5 expression was shown to be associated with resistance to M-tropic HIV-infection in $CD4^+$ T cells after stimulation with anti-CD28 antibody. In contrast, activation of T cells and natural killer cells with IL-2 led to enhanced CCR5 expression suggesting that ICER-mediated regulation of CD28RE in the context of CCR5 and/or IL-2 promoters could play an important role in modulation of CCR5 expression. Moreover, IFN-γ increases expression of chemokine receptors CCR1, CCR3, and CCR5, but not CXCR4 in monocytoid U937 cells. Collectively these data indicate that ICER-mediated downregulation of IL-2 and IFN-γ expression in T lymphocytes may have important consequences for CCR5 expression in cells of lymphoid as well as myeloid origin. (Bodor, J., Bodorova, J., Feigenbaum, L., Barabitskaja, O., Reitz, M., Gallo, R.C., Gress, R.E., 1999. *ICER represses MIP-1α and MIP-1β expression in stimulated subsets of human T cells: possible role of ICER in cAMP-mediated downregulation of CCR5 expression in monocyte-derived macrophages*. **Journal of Human Virology 2: 198, 1999**)

MHC class I expression is regulated dynamically in response to external stimuli. For example thyroid cells decrease MHC class I expression and transcription when stimulated by thyroid stimulating hormone (TSH), providing an excellent model for studying the dynamic modulation of transcription of MHC class I genes. PKA, a downstream effector of the cAMP pathway, reproduces the effects of TSH in repressing class I transcription. Repression is mediated by ICER, a transcriptional repressor induced by cAMP/PKA. ICER reduces class I promoter activity when introduced into FRTL-5 thyroid cells in the absence of TSH/cAMP. PKA/cAMP mediated repression of transcription involves multiple interacting upstream response elements in the class I promoter: a CRE-like element and at least two elements within a 30 bp segment, which overlaps with the interferon regulatory element. ICER binds to both the CRE-like element and the upstream 30 bp segment, generating a novel TSH-induced ternary complex. The present studies demonstrate that TSH mediated repression of class I transcription is the result of integrating signals from transcription factors through the higher order interactions of multiple regulatory elements. (Kirshner, S., Palmer, L., Bodor, J., Saji, M., Kohn, L.D., Singer, D.S. *Major histocompatibility class I gene transcription in thyrocytes: a series of interacting regulatory DNA sequence elements mediate thyrotropin/cyclic adenosine 3', 5'-monophosphate repression.* **Mol. Endocrinology 14: 82-98, 2000)**

The inducible cAMP early repressor (ICER) is likely to be involved in modulation of T cell responsiveness by its capacity to transcriptionally attenuate interleukine-2 (IL-2) gene expression. It seems clear that the failure to produce IL-2 is an important determinant of induction of anergy. Importantly, the CD28-responsive element (CD28RE), a composite DNA binding element consisting of NFAT and cyclic AMP responsive (CRE)-like motifs in position of (–160) of IL-2 promoter has the high affinity for ICER binding as well as NFAT/ICER complex formation. Moreover, CD28RE with adjacent DNA sequences was also shown to be essential for conferring anergy in T lymphocytes. Because ICER does not possess a transactivation domain required for the recruitment of CBP/p300, the binding of ICER to CD28RE and/or composite motifs containing CRE-like DNA motifs may lead to uncoupling of CBP/p300 thus extinguishing IL-2 expression as well as expression of numerous other cytokines and chemokines. (Bodor, J., Bodorova, J. Gress, R.E. *Suppression of T cell function: A potential role for transcriptional repressor ICER* **Journal of Leukocyte Biology, Review 67: 774-779, 2000).**

Depending on the nature of the costimulation in T lymphocytes, expression of the regulatory cytokines and chemokines is either susceptible or resistant to cyclic AMP (cAMP)-mediated inhibition. Here we demonstrate that forskolin, a cAMP-elevating agonist, inhibits early expression of interleukin-4 (IL-4), IL-5, IL-13, and interferon-γ (IFN-γ) in human peripheral blood T lymphocytes polarized toward T

helper 1 (Th1)- and T helper 2 (Th2)-like phenotypes. Our data show that cAMP-mediated inhibition of endogenously expressed cytokines, characteristic for Th1 and Th2-like phenotypes, correlate with the induction of potent transcriptional repressor ICER (inducible cAMP early repressor) in both subsets of T cells activated under the conditions of suboptimal IL-2 expression. Importantly, T helper-specific expression of certain chemokines represented by macrophage inflammatory proteins MIP-1α and MIP-1β is also susceptible to cAMP-mediated transcriptional attenuation. To determine that ICER *per se*, rather than forskolin-mediated elevations of intracellular cAMP, is responsible for the observed inhibitory effect, we generated transgenic mice expressing ICER under the control of a lymphocyte-specific *lck* promoter. Upon stimulation, transgenic thymocytes overexpressing ICER exhibited reduced levels of IL-2 and IFN-γ and failed to express MIP-1α and MIP-1β genes. Splenic T cells from ICER transgenic mice showed a defective proliferation and a lack of mixed lymphocyte reaction (MLR) response, implying that ICER-mediated inhibition of cytokine and chemokine expression may play an important role in T cell inactivation. (Bodor, J. Feigenbaum, L., Bodorova, J., Bare, C., Reitz, M.S., Gress, R.E. *Transcriptional repressor ICER supresses T cell responsiveness via attenuation of cytokine and chemokine gene expression.* **Journal of Leukocyte Biology 69: 1053-1069**, **2001).**

Under some conditions, the engagement of antigen receptor initiates apoptosis of T lymphocytes through the induced expression of Fas ligand (FasL). Forskolin, an activator of the cAMP/PKA pathway, results in antagonism of Fas-dependent, activation-induced cell death (AICD) by suppressed expression of the FasL. Forskolin mediated induction of ICER correlates with transcriptional attenuation of FasL expression in the AICD model 2B4 T cell hybridoma. ICER is easily inducible in human peripheral blood (PBL) T lymphocytes. In contrast, in B cells, its induction appears to be less responsive to forskolin treatment. Remarkably, ICER is present prior to forskolin treatment in a freshly isolated human PBL NK cells. Importantly, increased expression of ICER correlates with decreased FasL expression in both T and NK cells. ICER binds to the proximal nuclear factor of activated T cells (NFAT) DNA binding site of the FasL promoter, represented by one of the two NFAT motifs essential for NFAT-mediated FasL expression. In the presence of the minimal NFAT DNA-binding domain, the proximal NFAT motif allows ICER and NFAT to form an NFAT/ICER ternary complex in vitro. Moreover, in the activated 2B4 T cell hybridoma, the proximal NFAT motif participates in the downregulation of the FasL promoter mediated by ICER. These findings provide further insight into the mechanism involved in cAMP-mediated transcriptional attenuation of inducible FasL expression in T and NK lymphocytes. (Bodor, J., Bodorova, J., Bare, C., Hodge, D., Young, H.A., Gress, R.E., *Differential inducibility of the transcriptional repressor ICER and its role in modulation of Fas ligand expression in T and NK lymphocytes.***European Journal of Immunology 32: 203-212, 2002*)*

Local production of MIP-1β, a β-chemokine that blocks entry of HIV-1 into $CD4^+CCR5^+$ responder cells, seems to be an important factor in resistance to HIV-1 infection, and may contribute to control of local viral spread. The mechanisms governing MIP-1β expression in peripheral blood T cells are not clearly understood. Our results suggest that MIP-1β expression is mediated by both positive and negative transcription factors in human primary T cells. Based on our analysis the most important *cis*-acting elements are located in the proximal region of human MIP-1β promoter within a 192-bp region 5' to the TATA box. Cyclic AMP (cAMP) response element (CRE) at position –73 to –66 bp is an essential *cis*-regulatory element and represents a target for DNA binding of a potent transcriptional repressor termed inducible cAMP early repressor (ICER). Here, we present the evidence that a potent transcriptional repressor ICER binds to overlapping CRE and AP-1 sites and suppresses human MIP-1β promoter activity in complexes with yet to be identified proteins. We propose that formation of these inhibitory complexes could be governed by the availability of nuclear protein(s) that interact with ICER. These findings provide further insight into the mechanism involved in cAMP-mediated transcriptional attenuation of MIP-1β expression in human T cells. (Barabitskaja, O., Foulke, J.S., Pati, S., Bodor, J., Reitz, M.S., *Suppression of human MIP-1β transcription by inducible cAMP early repressor ICER.* **Journal of Leukocyte Biology 79: 378-387, 2006).**

HIV-1 infection of human monocytes induces production of prostaglandin E_2 (PGE_2) *in vitro* and *in vivo*, which has capacity to make the bystander T cells unresponsive (anergic). ICER, a potent transcriptional repressor induced by PGE_2 leads to transcriptional attenuation of interleukin-2 (IL-2) and an induction of T cell suppression. We propose that this HIV-1 triggered mechanism may subvert physiologically relevant suppression of IL-2 by regulatory $CD4^+CD25^+$ T (Treg) cells. Our preliminary studies suggest that the suppressive mechanism conveyed by Treg cells stems from coexpression of

ICER and Foxp3, which inhibits IL-2 expression. Moreover, naïve $CD4^+CD25^-$ responder T cells retrovirally transduced with Foxp3 can induce the accumulation of ICER and replace natural Treg cells. Importantly, ICER expression is induced in activated responder T cells early and correlates with a sharp decrease of IL-2 expression. Our preliminary studies indicate that ICER inhibits HIV-1 LTR expression and binds the cyclic responsive element (CRE)-like motifs termed DSE positioned downstream of transcription initiation site of HIV-1 LTR. Moreover, in monocytes PGE_2 induced transcriptional attenuation of CCR5 expression tightly correlates with upregulation of ICER. We conclude that ICER is a critical component of Treg-mediated inhibitory function that affects transcriptional attenuation of IL-2 production in T cells and that HIV-1 subverts this mechanism via PGE_2 and/or Tat-mediated induction of ICER. Bodor, J., *Regulation of HIV-1 and IL-2 transcription by inducible cAMP early repressor (ICER)*. **Retrovirology, 3: (Supplement I: S81, 2006).**

How regulatory T (T_R) cells dampen T cell responses remains unclear. Multiple modes of action have been proposed, including cell contact-dependent and/or cytokine-dependent mechanisms. Suppression may involve direct contact between T_R cells and responder T cells. Alternatively, T_R cells may act on dendritic cells (DCs) to reduce their ability to prime T cells by inducing the secretion of suppressive cytokines or the decrease of tryptophan metabolism. Here we review emerging novel mechanisms involved in contact-dependent T_R mediated suppression of interleukin-2 (IL-2) production in responder $CD25^-$ T lymphocytes and the potential involvement of *i*nducible *c*AMP *e*arly *r*epressor (ICER) in this suppression. Finally, cytokines such as transforming growth factor-β (TGF-β) and interleukin-10 (IL-10), produced by T_R cells or other cells, may exert local suppression, which can be conveyed by basic mechanism(s) acting in similar fashion as contact-dependent T_R mediated suppression. (Bodor, J., Fehervari, Z., Diamond, B., Sakaguchi, S., *Regulatory T cell mediated suppression; potential role of ICER.* **Journal of Leukocyte Biology, Review, 81: 161-167, 2007.**)

Here we report that *i*nducible *c*AMP *e*arly *r*epressor/*c*AMP *r*esponse *e*lement *m*odulator (ICER/CREM) is induced early in $CD25^+CD4^+$ T (T_R) assays mainly in activated $Foxp3^-$ effector T cells and this induction correlates with sharp decrease in number of IL-2 expressing T cells. Importantly, RNAi targeting of ICER/CREM in responder $CD25^-CD4^+$ T cells antagonizes T_R-mediated suppression. Moreover, forced expression of Foxp3 in naïve $CD25^-$ T cells induces constitutive expression of ICER/CREM in T cells with a regulatory phenotype. Foxp3 facilitates expression of ICER/CREM both in Foxp3 transductants as well as $CD25^-$ responder T cells suggesting that induction of T_R function in suppression assays may utilize contact-dependent interaction. Indeed, CTLA-4 blockade or use of B7-deficient $CD25^-$ responder T cells prevents ICER/CREM accumulation and leads to the rescue of IL-2 expression. Therefore, we propose that CTLA-4 binding to B7 ligands expressed on activated ligand-bearing $Foxp3^-$ effector T cells results in ICER/CREM-mediated transcriptional attenuation of IL-2. Collectively, these data suggest that Foxp3 expression in T_R cells imposes suppression in contact-dependent fashion by induction of constitutive ICER/CREM expression in activated $CD25^+$ $Foxp3^-$ T cell effectors thus preventing them from producing IL-2. (Bodor, J., Fehervari, Z., Diamond, B., Sakaguchi, S. *ICER/CREM-mediated transcriptional attenuation of IL-2 and its role in suppression by regulatory T cells.* **European Journal of Immunology, 37: 884-895, 2007.**)

There is growing evidence that cyclic adenosine monophosphate (cAMP) is directly involved in suppression by naturally occurring regulatory T cells (Treg cells). The *i*nducible *c*AMP *e*arly *r*epressor (ICER) is expressed in Treg cells as well as in effector $CD4^+$ T cells. In CD3/CD28-activated effector $CD4^+$ T cells transcriptional repressor ICER is sequestered to the cytoplasm thus unable to oppose NFAT-driven transcription. However, in Treg cells, ICER remains nuclear while NFAT fails to translocate into the nucleus even after CD3/CD28 re-stimulation. Importantly, upon administration of superagonistic CD28-specific monoclonal antibodies (CD28SA) *in vivo*, ICER is nuclear in effector $CD4^+$ T cells in the presence of Treg cells (no diphteria toxin treatment) but cytosolic in their absence (after diphteria toxin treatment) in "depletion of regulatory T cell" (DEREG) mice. Thus, naturally occurring Treg cells similarly to forskolin could enforce nuclear localization of ICER *in vivo.* (Vaeth, M., Gogishvili, T., Bopp, T., Klein, M., Berberich-Siebelt, F., Klein-Hessling, S., Schmitt, E., Huenig, T., Serfling, E., and Bodor, J.***** *Cyclic AMP-mediated shuffling of ICER alters NFAT-dependent suppression by regulatory T cells*. **2nd European Congress of Immunology 2009 (ed. R.E. Schmidt), pp. 137-143, Medimond International Proceedings, Bologna, Italy, 2009**).

Inducible cAMP early repressor (ICER) is a transcriptional repressor, which, because of alternate promoter use, is generated from the 3' region of the cAMP response modulator (*Crem*) gene. Its

expression and nuclear occurrence are elevated by high cAMP levels in naturally occurring regulatory T cells (nTregs). Using two mouse models, we demonstrate that nTregs control the cellular localization of ICER/CREM, and thereby inhibit IL-2 synthesis in conventional $CD4^+$ T cells. Ablation of nTregs in depletion of regulatory T-cell (DEREG) mice resulted in cytosolic localization of ICER/CREM and increased IL-2 synthesis upon stimulation. Direct contacts between nTregs and conventional $CD4^+$ T cells led to nuclear accumulation of ICER/CREM and suppression of IL-2 synthesis on administration of CD28 superagonistic (CD28SA) Ab. In a similar way, nTregs communicated with B cells and induced the cAMP-driven nuclear localization of ICER/CREM. High levels of ICER suppressed the induction of nuclear factor of activated T cell c1 (*Nfatc1*) gene in T cells whose inducible *Nfatc1* P1 promoter bears two highly conserved cAMP-responsive elements to which ICER/CREM can bind. These findings suggest that nTregs suppress T-cell responses by the cAMP-dependent nuclear accumulation of ICER/CREM and inhibition of NFATc1 and IL-2 induction. (Vaeth, M., Gogishvili, T., Bopp, T., Klein, M., Gattenloehner, S., Berberich-Siebelt, F., Avots, A., Sparwasser, T., Grebe, N., Schmitt, E., Hünig, T., Serfling, E., and Bodor, J.* *Regulatory T cells facilitate the nuclear accumulation of inducible cAMP early repressor (ICER) and suppress nuclear factor of activated T cell c1 (NFATc1).* **Proc. Natl. Acad. Sci. USA 108: 2480-2485, 2011)**.

Elevated levels of intracellular cyclic AMP (cAMP) in naturally occurring T regulatory (nTreg) cells play a key role in nTreg-cell – mediated suppression. Upon contact with nTreg cells, cAMP is transferred from nTreg cells into activated target $CD4^+$ T cells and/or antigen-presenting cells (APCs) via gap junctions to suppress CD4+ T cell function. cAMP facilitates the expression and nuclear function of a potent transcriptional inhibitor, *i*nducible *c*AMP *e*arly *r*epressor (ICER), resulting in ICER-mediated suppression of interleukin-2 (IL-2). Furthermore, ICER inhibits transcription of nuclear factor of activated T cells c1/α (NFATc1/α) and forms inhibitory complexes with preexisting NFATc1/c2, thereby inhibiting NFAT-driven transcription, including that of IL-2. In addition to its suppressive effects mediated via ICER, cAMP can also modulate the levels of surface-expressed cytotoxic T lymphocyte antigen 4 (CTLA-4) and its cognate B7 ligands on conventional CD4+ T cells and/or APCs, fine-tuning suppression. These cAMP-driven nTreg-cell suppression mechanisms are the focus of this review. (Bodor, J*., Bopp, T., Vaeth, M., Klein, M., Serfling, E., Hünig, T., Becker, C., Schild, H., and Schmitt, E. *Cyclic AMP underpins suppression by regulatory T cells.* **European Journal of Immunology 42: 1375-1384, 2012**).

Naturally occurring $CD4^+CD25^+Foxp3^+$ regulatory T cells (nTregs) emerge from the thymus as a distinct and mature population of T cells. However, the molecular mechanisms underlying their development and function are still elusive. Therefore, we performed DNA microarray analyses. Compared to murine and human $CD4^+CD25^-$ T cells, $CD4^+CD25^+Foxp3^+$ T cells highly express HELIOS, a transcription factor belonging to the IKAROS family. This result was verified by qRT-PCR as well as western blot. To study the role of HELIOS in nTreg development and function we analyzed the influence of HELIOS on the Foxp3 promoter by performing reporter gene assays. In contrast to the control vector, overexpression of HELIOS leads to a strong induction of the Foxp3 promoter. Furthermore, ChIP (chromatin immunoprecipitation) analyses revealed that HELIOS binds to the Foxp3 promoter. In addition, transfection of OVA-tg CD4+ T cells with HELIOS and transfer of these cells into RAG2KO mice resulted in the generation of $Foxp3^+$ T cells. To study the potency of these generated $Foxp3^+$ T cells, we tested HELIOS or control vector transfected OVA-tg $CD4^+$ T cells in a murine model of DTH resulting in a significant difference of footpad swelling. In summary, these data suggest that the transcription factor HELIOS might play an important role for the development and function of naturally occurring Tregs. Klein, M., Ullrich, N., Klein-Hessling, S., Becker, M., Bodor, J. Serfling, E., Bopp, T., Schmitt, E. *The role of IKAROS transcription factor HELIOS in nTreg development and function. In preparation.*