

# TGFβ induces murine CXCR5 and Bcl6 and differentially specifies Tfh versus Th17 cell fates

Y. Chang<sup>1,2</sup>, L. Bach<sup>1</sup>, M. Hasiuk<sup>3</sup>, C. Tsui<sup>4</sup>, L. Wen<sup>4</sup>, N. G. Melo<sup>1</sup>, D. T. Utzschneider<sup>4</sup>, T. Raj<sup>2</sup>, S. Heink<sup>5</sup>, T. Elmzzahi<sup>4,6</sup>, J. Cheng<sup>7</sup>, J. Zeiträg<sup>2</sup>, P. J. Jost<sup>8</sup>, D. Alterauge<sup>2</sup>, F. Dahlström<sup>2</sup>, J. C. Becker<sup>1</sup>, M. v. Uelft<sup>9</sup>, M. Becker<sup>10,11</sup>, S. Reschke<sup>12</sup>, S. Krebs<sup>12</sup>, H. Blum<sup>12</sup>, C. Ohnmacht<sup>13</sup>, C. Neumann<sup>14</sup>, J. Hasenauer<sup>8,15,16</sup>, F. Meissner<sup>7,17</sup>, M. Beyer<sup>6,11</sup>, T. Korn<sup>5,18</sup>, V. Heissmeyer<sup>2,19</sup>, A. Kallies<sup>4</sup>, L. T. Jeker<sup>3,20</sup>, D. Baumjohann<sup>1,2</sup>

<sup>1</sup>Medical Clinic III for Oncology, Hematology, Immuno-Oncology and Rheumatology, University Hospital Bonn, University of Bonn, Venusberg-Campus 1, 53127 Bonn, Germany; <sup>2</sup>Institute for Immunology, Faculty of Medicine, Biomedical Center, LMU Munich, Grosshaderner Str. 9, 82152 Planegg-Martinsried, Germany; <sup>3</sup>Department of Biomedicine, Basel University Hospital and University of Basel, Hebelstrasse 20, CH-4031 Basel, Switzerland; <sup>4</sup>The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria 3000, Australia; <sup>5</sup>Institute for Experimental Neuroimmunology, Technical University of Munich School of Medicine, 81675 Munich, Germany; <sup>6</sup>Immunogenomics & Neurodegeneration, Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Bonn, Germany; <sup>7</sup>Experimental Systems Immunology, Max Planck Institute of Biochemistry, Martinsried, Germany; <sup>8</sup>Faculty of Mathematics and Natural Sciences, University of Bonn, Bonn, Germany; <sup>9</sup>Genomics and Immunoregulation, Life & Medical Sciences (LIMES) Institute, University of Bonn, Bonn, Germany; <sup>10</sup>Systems Medicine, Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Bonn, Germany; <sup>11</sup>PRECISE Platform for Single Cell Genomics and Epigenomics, Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE) and the University of Bonn, Bonn, Germany; <sup>12</sup>Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU Munich, Feodor-Lynen-Str. 25, 81377 Munich, Germany; <sup>13</sup>Center of Allergy and Environment (ZAUM), Helmholtz Zentrum München and Technical University of Munich, Munich, Germany; <sup>14</sup>Department of Microbiology, Infectious Diseases and Immunology, Charité – Universitätsmedizin Berlin, Berlin, Germany; <sup>15</sup>Center for Mathematics, Technical University of Munich, Garching, Germany; <sup>16</sup>Institute of Computational Biology, Helmholtz Zentrum München, Neuherberg, Germany; <sup>17</sup>Department of Systems Immunology and Proteomics, Institute of Innate Immunity, Medical Faculty, University of Bonn, Germany; <sup>18</sup>Munich Cluster for Systems Neurology (SyNergy), 81377 Munich, Germany; <sup>19</sup>Research Unit Molecular Immune Regulation, Helmholtz Zentrum München, Feodor-Lynen-Str. 21, 81377 Munich, Germany; <sup>20</sup>Transplantation Immunology & Nephrology, Basel University Hospital, Petersgraben 4, CH-4031 Basel, Switzerland

## Abstract

T follicular helper (Tfh) cells are essential for effective antibody responses but deciphering the intrinsic wiring of mouse Tfh cells has long been hampered by the lack of a reliable protocol for their generation *in vitro*. We report that TGFβ induces robust expression of the Tfh hallmark molecules CXCR5 and Bcl6 in activated mouse CD4 T cells *in vitro* and that TGFβ-induced mouse CXCR5-positive Tfh cells are functional and provide critical help to B cells in a contact-dependent manner. Detailed dissection of the TGFβ-induced molecular pathways revealed that CXCR5 expression is independent of Bcl6 and that excess IL-2 in high-density T cell cultures interferes with the TGFβ-induced Tfh cell program. Notably, classical TGFβ-induced Th17 cultures also yield separate CXCR5-positive and IL-17A-producing cells, thus highlighting shared and distinct cell fate trajectories of Tfh and Th17 cells and thereby underscoring the pleiotropic functions of TGFβ in T helper cell biology.