The Menin–Bach2 axis is critical for regulating CD4 T-cell senescence and cytokine homeostasis

Makoto Kuwahara, Junpei Suzuki, Soichi Tofukuji, Takeshi Yamada, Makoto Kanoh, Akira Matsumoto, Saho Maruyama, Kohei Kometani, Tomohiro Kurosaki, Osamu Ohara, Toshinori Nakayama, & Masakatsu Yamashita

Although CD4 T-cell senescence plays an important role in immunosenescence, the mechanism behind this process remains unclear. Here we show that T cell-specific Menin deficiency results in the premature senescence of CD4 T cells, which is accompanied by the senescence-associated secretory phenotype after antigenic stimulation and dysregulated cytokine production. Menin is required for the expansion and survival of antigen-stimulated CD4 T cells in vivo and acts by targeting Bach2, which is known to regulate immune homeostasis and cytokine production. Menin binds to the Bach2 locus and controls its expression through maintenance of histone acetylation. Menin binding at the Bach2 locus and the Bach2 expression are decreased in the senescent CD4 T cells. These findings reveal a critical role of the Menin-Bach2 pathway in regulating CD4 T-cell senescence and cytokine homeostasis, thus indicating the involvement of this pathway in the inhibition of immunosenescence.

DOI: 10.1038/ncomms4555

1 Department of Immunology, Graduate School of Medicine, Ehime University, Shitsukawa, Toon, Ehime 791-0295, Japan. 2 Translational Research Center, Ehime University Hospital, Shitsukawa, Toon, Ehime 791-0295, Japan. 3 Laboratory of Medical Genomics, Department of Human Genome Research, Kazusa DNA Research Institute, Kisarazu, Chiba 292-0818, Japan. 4 Department of Infection and Host Defenses, Graduate School of Medicine, Ehime University, Shitsukawa, Toon, Ehime 791-0295, Japan. 5 Laboratory for Lymphocyte Differentiation, WPI Immunology Frontier Research Center, Osaka University, Suita, Osaka 565-0871, Japan. 6 Department of Immunology, Graduate School of Medicine, Chiba University, Chuo-ku, Chiba 260-8670, Japan. 7 Department of Immunology, Graduate School of Medicine, Chiba University, Chuo-ku, Chiba 260-8670, Japan. 8 CREST, Japan Science and Technology Agency, Chuo-ku, Chiba 260-8670, Japan. 9 PRESTO, Japan Science and Technology Agency, Ehime University, Shitsukawa, Toon, Ehime 791-0295, Japan. Correspondence and requests for materials should be addressed to M.Y. (email: yamamasa@m.ehime-u.ac.jp).
Age-induced alterations of innate and adaptive immunity are generally viewed as detrimental and designated as immunosenescence. Immunosenescence particularly affects the T-cell compartment and is involved in the age-related decline of immune functions, which increase the susceptibility of elderly individuals to infectious diseases and certain cancers. Further, immunosenescence induces a pro-inflammatory state and increases the susceptibility to autoimmune diseases such as rheumatoid arthritis. Evidence indicates that a prominent effect of ageing on immunity is reduced humoral responses, and that ageing is accompanied by alterations of the CD4 T-cell immunity. Therefore, understanding immunosenescence requires knowledge of the age-associated alterations of CD4 T-cell functions and induction of cellular senescence.

CD4 T-cell senescence represents a subset of cellular senescence, which is characterized by irreversible proliferation arrest caused by oxidative stress, reactive oxygen species, oncogene activity or the inactivation of tumour suppressor genes. These factors contribute to tumour-suppression, wound healing and ageing. Senescent cells can significantly harm the tissue microenvironment through the acquisition of a senescence-associated secretory phenotype (SASP), which is characterized by a striking increase in the secretion of pro-inflammatory cytokines, chemokines, matrix remodelling factors and pro-angiogenic factors. These factors deleteriously alter tissue homeostasis, leading to chronic inflammation and cancer.

Certain germinal mutations of MEN1, which encodes MENIN, cause multiple endocrine neoplasia type 1 (ref. 15), which is an autosomal-dominant syndrome characterized by concurrent parathyroid adenomas, gastroenteropancreatic tumours and several other tumour types. Menin does not share significant amino-acid sequence similarity with other proteins, but it possesses nuclear localization signals within its C terminus. Menin interacts with H3K4 methyltransferases, including mixed-lineage leukaemia 1 (MLL1), and it is an oncogenic cofactor for MLL-associated leukemogenesis. Also, Menin is known to associate with a trihelix family histone methyltransferase complex and contributes to the DNA damage response and cell cycle regulation. Paradoxically, Menin recruits polycomb repressive complex 2 (PRC2) at the p53/TP53 locus, and its loss abrogates the binding of the PRC2 components enhancer of zeste homologue 2 (EZH2) and SUZ12 to PTN in lung adenocarcinoma cells. Further, Menin associates with the JunD proto-oncogene product (JUND), nuclear factor of kappa light poly peptide gene enhancer in B cells (NF-κB), peroxisome proliferator-activated receptor gamma (PPAR-γ), SMAD family member 3 (SMAD3) and β-catenin, indicating its involvement in transcriptional activation and repression.

Bach2 (BTB and Cap’n’collar (CNC) homology 1; basic leucine zipper transcription factor 2) belongs to the CNC gene family. Bach2-deficient mice with CD4-Cre transgenic (TG) mice. As previously reported, the T-cell numbers were moderately decreased in the spleen and mesenteric lymph node of Menin KO mice (Supplementary Fig. 1a). Menin KO naive CD4 T cells expressed wild-type (WT) levels of interleukin (IL)-2 mRNA after T-cell receptor (TCR) stimulation (Supplementary Fig. 1b). IL-4 and interferon (Ifn)-γ mRNAs were not detected in either WT or Menin KO naive CD4 T cells (Supplementary Fig. 1b). The early-phase cell division (days 1–3) induced by TCR stimulation was enhanced in the Menin KO naive CD4 T cells (Supplementary Fig. 1c).

First, we assessed the role of Menin in CD4 T-cell growth in vitro. Although Menin KO naive CD4 T cells normally expanded after stimulation, the growth rate was reduced after day 4, even in the presence of exogenous IL-2 (Fig. 1a). To assess the effects of Menin deficiency on the cell cycle, we measured the percentage of replicating cells after incubation with 5-ethynyl-2′-deoxyuridine (EdU). While approximately 50% of the WT CD4 T cells were EdU positive, indicating replication (S phase), only approximately 30% of Menin KO CD4 T cells were positive 7 days after the initial TCR stimulation (Fig. 1b). A reduced number of EdU-positive cells was also detected in the Menin KO CD4 T-cell cultures on days 3 and 5 (Supplementary Fig. 2a). Increased expression of cyclin-dependent kinase (CDK) inhibitors, Cdk1a, Cdkn2a and Cdkn2b, was detected in the Menin KO CD4 T cells (Fig. 1c). Cell death was increased in the Menin KO CD4 T-cell cultures (Annexin V positive: approximately 40%) compared with that in WT cultures (Annexin V positive: approximately 20%) (Fig. 1d). Increased cell death was detected in the Menin KO CD4 T-cell cultures starting 3 days after the initial stimulation (Supplementary Fig. 2b). There was also increased expression of the mRNA encoding pro-apoptotic factors, such as p19Arf, TP53 apoptosis effector (Perp) and phorbol-12-myristate-13-acetate-induced protein 1 (Pmaip1) (Fig. 1e), indicating that the reduced number of Menin KO CD4 T cells after day 4 was caused by reduced cell cycle progression and an increased rate of cell death.

Early cell cycle arrest accompanied by the increased expression of Cdk inhibitors is a hallmark of cellular senescence, suggesting that premature senescence is induced after TCR stimulation of Menin KO CD4 T cells. Although the Menin-deficient naive CD4 T cells did not express a senescence-associated β-galactosidase (SA β-Gal) activity, a marker of cellular senescence, the proportion of SA β-Gal-positive cells was increased in the Menin KO CD4 T-cell cultures 3 days after TCR-mediated stimulation (Supplementary Fig. 2c). A strong expression of the SA β-Gal...
Figure 1 | Menin deficiency induces premature CD4 T-cell senescence. (a) The TCR-induced proliferation of the Menin KO naive CD4 T cells in the presence or absence of IL-2. The average of three independent cultures and s.d. are shown. (b) The results of the cell cycle analysis of CD4 T cells from the Menin KO and WT mice cultured under IL-2 conditions for 7 days. The percentages of G0/G1- and S-phase cells in three independent cultures are shown (right). (c) The results of the quantitative reverse transcriptase polymerase chain reaction (RT-PCR) analysis of mRNA encoding Cdk inhibitors (vertical axes) in the WT and Menin KO TH cells. The results represent the expression levels relative to Hprt with the s.d. (d) The results of the cell death analysis of CD4 T cells from the WT and Menin KO mice on day 7. The percentages of annexin V-positive cells in three independent cultures are shown (right). (e) The results of the quantitative RT-PCR analysis of mRNAs encoding pro-apoptotic proteins (vertical axes) in the WT and Menin KO TH cells cultured under IL-2-conditions for 7 days (left). The percentages of annexin V-positive cells in three independent cultures are shown (right). (f) The phosphorylation of RelA (pSer534) in the WT and Menin KO TH cells cultured for 7 days. (g) The phosphorylation of RelA (Ser534) in the WT and Menin KO T\textsubscript{el} cells cultured for 7 days. (h) Assay of the binding of RelA and RelB to an NF-κB-binding consensus motif in the WT and Menin KO T\textsubscript{el} cells cultured under IL-2-conditions for 7 days. Four (panel c and e) and three (panels a,b,d,f,g and h) independent experiments were performed. *P<0.01, **P<0.01 (Student’s t-test).

The role of Menin in CD4 T response in vivo. To investigate the role of Menin in CD4 T-cell expansion and survival in vivo, we monitored antigen-specific CD4 T cells following immunization in an adoptive transfer model. Naive BALB/c mice were transplanted with the Menin KO or WT naïve CD4 T cells and immunized with ovalbumin (OVA) plus lipopolysaccharide (LPS). After 4 days, the number of transplanted Menin KO cells was approximately 25% of that of the WT cells, and the number decreased further on day 7, and the cells did not accumulate in the spleen during the memory phase (day 50) (Fig. 2a).

To further examine the role of Menin in the survival of CD4 T cells after antigenic stimulation, in vitro-activated effector CD4 T cells from WT (CD45.1) and Menin KO (CD45.2) mice were mixed 1:1 and adoptively transferred into naïve TCR-β\textsuperscript{+} KO mice. As shown in Fig. 2b, significant reductions of CD45.2\textsuperscript{+} positive Menin KO CD4 T cells were detected in all the tested tissues (the spleen, liver, lung and PBMCs) 4 weeks after the cell transfer.

Next, we investigated the airway inflammation in Menin KO mice using an in vivo model. WT and Menin KO mice were immunized with OVA containing alum and then were challenged with inhaled OVA. This decreased the inflammatory cell infiltration in the bronchioalveolar lavage (BAL) fluid of the OVA-immunized Menin KO mice compared with that in WT mice (Fig. 2c). The number of mononuclear cells infiltrating the perilbronchiolar regions of the lungs in Menin KO mice also decreased (Fig. 2d).
Finally, we assessed the impact of Menin deficiency on the host defenses against *Listeria monocytogenes* (Lm) infection. The bacterial burden in the spleens of the Menin KO mice 3 days after primary infection was comparable to that observed in the WT mice (Fig. 2e, left panel). However, the bacterial burden was significantly higher in the Menin KO mice than in the WT mice after secondary Lm infection (recall response) (Fig. 2e, right panel). These results indicate that the expansion, survival and function of antigen-stimulated CD4 T cells in vivo require Menin.

Menin-deficient CD4 T cells exhibit the SASP phenotype. Decreased cell-surface expression of CD27 and CD62L and increased expression of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) are characteristics of exhausted and senescent T cells.\(^{40-42}\) The numbers of CD27\(^{low}\)/CD62L\(^{low}\) and CD27\(^{hi}\)/CD62L\(^{hi}\) cells were markedly increased in the Menin KO CD4 T-cell cultures compared with those observed in the WT-cell cultures (Fig. 3a and Supplementary Fig. 3a). In addition, moderate upregulation of CTLA-4 was detected in the Menin KO T\(_H\) cells, the level of which was elevated following immobilized anti-TCR-\(\beta\) mAb restimulation (Supplementary Fig. 3b).

The SASP phenotype is characterized by the secretion of pro-inflammatory factors, including IL-6, IL-8 and pro-inflammatory chemokines,\(^{10,11}\) and senescent CD4 T cells produce a high level of osteopontin (OPN),\(^{43}\) We found that the number of IL-6- and OPN-producing cells markedly increased in the Menin KO CD4 T-cell cultures, while the number of IL-2-producing cells was marginally decreased (Fig. 3b). Increased production of IL-6 and OPN in the Menin KO T\(_H\) cells was detected using enzyme-linked immunosorbent assays (ELISAs) (Supplementary Fig. 3c). The production of pro-inflammatory chemokines, chemokine (C-C motif) ligand 3 (CCL-3), CCL-4, CCL-5 and chemokine (C-X-C motif) ligand 2 (CXCL-2), increased in the Menin KO T\(_H\) cells (Fig. 3c).

Using a DNA microarray analysis, we found that the expression of a series of pro-inflammatory factors was upregulated in the Menin KO T\(_H\) cells compared with that in the WT cells (Supplementary Data 1). For example, the expression of genes encoding the components of cytotoxic granules (*Gzm a, b, c, d, e, f, g* and *Prf1*), which are involved in age-related cardiovascular and pulmonary diseases,\(^{44}\) markedly increased (Fig. 3d). The augmented expression of pro-inflammatory enzymes such as *Adam8, Alox5, Casp1, Ctsd* and *Ctsb* was detected in the Menin KO T\(_H\) cells (Supplementary Fig. 3d). The increased production of pro-angiogenic factors is another hallmark of SASP in senescent cells.\(^{10,45}\) The expression of genes encoding pro-angiogenic factors, such as *Esm1, Pdgfa*,...
experiments were performed with TH cells cultured under IL-2 conditions for 5 days. At least three independent experiments (analysis of IL-4 and IFN-γ on the cell surface of the WT and Menin KO TH cells. The cell numbers are indicated in each quadrant. (lower). (Supplementary Fig. 3e). These results strongly suggest that Menin deficiency causes the SASP in the effector CD4 T cells.

Figure 3 | Menin-deficient effector CD4 T cells exhibit a senescence-associated secretory phenotype. (a) The staining profile of CD62L/CD27 on the cell surface of the WT and Menin KO TH cells. The cell numbers are indicated in each quadrant. (b) The results of the intracellular FACS analysis of IL-2/IL-6, or IL-2/OPN in the WT and Menin KO TH cells. The percentages with s.d. of IL-2-, IL-6- and OPN-producing cells in three independent cultures are shown (lower). (c) The ELISA of chemokines in supernatants of the cells in b. (d) The results of the quantitative RT-PCR analysis of Gzm's and Prf1 in cells in b. The results are presented relative to the expression of Hprt mRNA with the s.d. (e) The results of the intracellular FACS analysis of IL-4 and IFN-γ in cells cultured under IL-2 (top) or neutral-conditions (bottom). The numbers of cells are indicated in each quadrant. (f) The ELISA of cytokines in supernatants of the cells cultured under IL-2 conditions and restimulated with an immobilized anti-IFN-γ for 16 h. All experiments were performed with TH cells cultured under IL-2 conditions for 5 days. At least three independent experiments (a-f) were performed. **P<0.01 (Student’s t-test).

S100a4 and Vegfc, upregulated in the Menin KO TH cells (Supplementary Fig. 3e). These results strongly suggest that Menin deficiency causes the SASP in the effector CD4 T cells.

Cytokine productions in Menin-deficient CD4 T cells. Changes in the cytokine production profile of senescent CD4 T cells have been previously reported. Next, we analysed the cytokine productions in Menin KO effector CD4 T cell in vitro. The generation of IFN-γ and IL-4-producing cells was increased in the Menin KO naive CD4 T cell cultured under IL-2 conditions compared with that observed in the WT cells (Fig. 3e upper panel and Supplementary Fig. 4a). Furthermore, IL-4/IFN-γ-double producers were induced in Menin KO CD4 T-cell cultures (23.2%) but not in WT cultures (2.5%) (Fig. 3e upper panel and Supplementary Fig. 4a). Enhanced generation of IFN-γ-producing cells was detected even in the presence of an anti-IFN-γ mAb, whereas the generation of IL-4-producing cells was blocked by an IL-4-neutralizing antibody (Fig. 3e lower panel, Supplementary Fig. 4a,b). The production of Th12-related cytokines, such as IL-5, IL-10, and IL-13, was increased in the Menin KO TH cells (Fig. 3f), while the IL-9, IL-17A and tumour necrosis factor (TNF-α) production levels remained unaffected under IL-2 conditions (Supplementary Fig. 4c).

To assess the responsiveness of the Menin KO naive CD4 T cells to IL-4 and IL-12, we stimulated naive CD4 T cells with anti-TCR-β and anti-CD28 Ab in the presence of various doses of IL-4 or IL-12. As we previously reported, the Menin-deficient CD4 T cells normally differentiated into IL-4-producing cells under optimal Th12 culture conditions (Supplementary Fig. 4d, right panel). Compared with WT cells, the Menin KO naive CD4 T cells readily differentiated into IL-4-producing cells in response to low concentrations of IL-4 (Supplementary Fig. 4d). Enhanced generation of IFN-γ-producing cells was detected in the Menin KO CD4 T cells even in the presence of IL-4 (Supplementary Fig. 4d). The IL-12-dependent induction of IFN-γ-producing cells was augmented in the Menin KO naive CD4 T-cell cultures, although the Menin-deficient CD4 T cells normally differentiated into IFN-γ-producing cells under optimal Th1 culture conditions (Supplementary Fig. 4e). These results indicate the crucial role of Menin in regulating the IFN-γ and Th12 cytokine production in CD4 T cells under the condition with limited concentration of IL-4 or IL-12, respectively.
Bach2 is a downstream target of Menin. Next, we investigated the molecular mechanisms involved in the induction of SASP and cytokine production in the Menin-deficient cells. On the basis of the results of the DNA microarray analysis, we focused on Bach2, which was differentially expressed in the Menin KO T\textsubscript{H} cells (Supplementary Data 1). The Bach2 mRNA (Fig. 4a, right) and protein levels (Fig. 4b) were significantly reduced in the Menin KO T\textsubscript{H} cells compared with those in the WT cells. The Bach2 mRNA level in the Menin KO naive CD4 T cells was marginally decreased (Fig. 4a, left). Next, we transduced Bach2 into the Menin KO T\textsubscript{H} cells. The Bach2 protein levels in the Bach2-transduced T\textsubscript{H} cells were comparable to those observed in the WT T\textsubscript{H} cells (Supplementary Fig. 5a). The induction of IL-4- and IFN-\(\gamma\)-producing cells from the Menin KO naive CD4 T cells was normalized by the transduction of Bach2 (Fig. 4c). The transduction of the Menin KO T\textsubscript{H} cells with Bach2 restored the enhanced mRNA expression levels of Gzm's and Prf1 (Fig. 4f), Clt4 (Supplementary Fig. 5b) and the genes that encode pro-inflammatory enzymes and pro-angiogenic factors (Supplementary Fig. 5c).

We crossed Bach2\textsuperscript{lox/lox} mice\textsuperscript{28} with CD4-Cre TG mice and generated T-cell-specific Bach2-deficient (Bach2 KO) mice to confirm the roles of Bach2 in CD4 T-cell senescence and cytokine production. Although the number of T cells was moderately reduced (Supplementary Fig. 6a), a significant number of naive CD4 T cells remained in the spleens of the Bach2 KO mice (Supplementary Fig. 6b). The generation of IL-4- and IFN-\(\gamma\)-producing cells was accelerated, and IL-4/IFN-\(\gamma\)-double producers were generated in the Bach2 KO naive CD4 T cells cultured under IL-2 conditions (Fig. 5a, upper panel). The augmentation of IL-6-producing cells was observed in Bach2 KO T\textsubscript{H} cells (Fig. 5a, lower). The levels of IL-5, IL-10, IL-13, OPN and pro-inflammatory chemokines were increased in the Bach2 KO T\textsubscript{H} cells compared with those in WT cells (Fig. 5b). Moreover, the enhanced expression of Gzm's and Prf1 (Fig. 5c), pro-inflammatory factors (Supplementary Fig. 7a) and pro-angiogenic factors (Supplementary Fig. 7b) was detected in Bach2 KO T\textsubscript{H} cells. The mRNA for Clt4 was also upregulated in the Bach2 KO T\textsubscript{H} cells (Supplementary Fig. 7c). The expression of the SA \(\beta\)-Gal
activity was increased in the Bach2 KO TH cells (Fig. 5d).
Furthermore, the DNA-binding activity of RelA was upregulated in the Bach2 KO TH cells (Fig. 5e). These data indicate that the senescent phenotypes of Bach2 KO TH cells are similar to those of Menin KO cells.

Menin maintains histone acetylation at the Bach2 locus. To investigate the molecular mechanism by which Menin controls the Bach2 expression, we assessed changes in the histone modification of Bach2 in the Menin KO TH cells using a ChIP-seq analysis. The level of H3K4me3 at Bach2 peaked at the transcriptional start site (Tss) and was slightly decreased in the Menin KO TH cells compared with that in the WT TH cells (Fig. 6a, upper). The level of H3K27me3 at the Tss of Bach2 was increased in the Menin KO TH cells (Fig. 6a, middle). Furthermore, the level of acetylated (ac) histone H3K27 was markedly reduced in the Menin KO TH cells (Fig. 6a, lower). We further compared the histone modifications around the Tss of Bach2 between Menin KO and WT CD4 T cells. A schematic representation of the region surrounding the Tss of Bach2 and the location of the amplicons used in ChIP-qPCR are shown in Supplementary Fig. 8a. Decreased levels of H3K4me3 and H3K27ac and an increased level of H3K27me3 around the Tss of Bach2 were detected in the Menin KO TH cells cultured under IL-2 conditions for 5 days (Fig. 6b). Interestingly, a striking reduction in the H3K27ac level was detected in the Menin KO TH-cell cultured for 3 days, while the H3K4me3 level remained unchanged (Supplementary Fig. 8b). The level of H3K4me3 was also unaffected in the Menin KO naive CD4 T cells (Supplementary Fig. 8b). In addition, we were able to detect only a small reduction in the H3K27ac level, as well as upregulation of H3K27me3, in the Menin KO naive CD4 T cells (Supplementary Fig. 8b). These results suggest that the reduction of H3K27ac observed after TCR stimulation has the primary effect of inducing Menin deficiency.

Menin is a component of the KMT2A/2B complex, which is involved in the methylation of histone H3K4, an active histone marker. We assessed changes in the binding of KMT2A/2B and PRC2 components at Bach2 in the Menin KO TH cells using ChIP-qPCR. As shown in Fig. 6c, the binding of the KMT2A/2B complex, including that with MLL1, Rbpb5 and Wdr5, was decreased in the Menin KO TH cells, whereas the binding of Ezh2 and Suz12 (PRC components) was augmented (Fig. 6c). However, the level of binding of the KMT2A/2B complex with the Bach2 gene was relatively low.

Next, we treated Menin KO TH cells with trichostatin A (TSA), an HDAC inhibitor, to determine the role of histone acetylation in maintaining Bach2 transcription. The Bach2 expression in the Menin KO TH cells was completely restored by TSA treatment (Fig. 6d). The recruitment of RNA polymerase II and the
All experiments were performed with TH cells cultured under IL-2 conditions for 5 days. Bach2 to the pAb, and immunoblotted with anti-Menin pAb. Two (shown. (b) The results of the ChIP assay with a quantitative PCR analysis of the histone modification (H3K4me3, H3K27me3 and H3K27ac) status around the Tss of the Bach2 locus in the WT and Menin KO TH cells. The relative intensity (input) with the s.d. is shown. (c) ChIP with a quantitative PCR analysis of the binding of HMT2A/B (MLL1, Rbbp5 and Wdr5) and PRC2 (Ezh2 and Suz12) components to the Bach2 Tss region in the Menin KO TH cells. (d) The results of the quantitative RT-PCR analysis of Bach2 mRNA expression in the Menin KO TH cells treated with TSA for 24 h. The results are presented relative to the mRNA expression of Hprt mRNA with the s.d. (e) The ChIP data with the results of the quantitative PCR analysis of PCAF binding to the Bach2 Tss region. The relative intensity (input) with the s.d. is shown. (f) The association between Menin and PCAF in the TH cells. The nuclear lysates (5 x 10⁶ cells) were precipitated with anti-PCAF mAb or anti-MLL1 pAb, and immunoblotted with anti-Menin pAb. Two (a) and three (b-f) independent experiments were performed. **P<0.01 (Student’s t-test). All experiments were performed with TH cells cultured under IL-2 conditions for 5 days.

H3K4me3 and H3K27ac status at the Tss of the Bach2 gene locus were also restored by TSA (Supplementary Fig. 8c). We detected enhanced binding of HDAC1 at the Bach2 locus in the Menin KO mice, and the level of which was reduced by TSA treatment (Supplementary Fig. 8d). To identify histone acetyltransferases that are recruited in a Menin-dependent manner, we performed a series of ChIP-qPCR assays and found that the binding of lysine acetyltransferase 2B (PCAF) around the Bach2 Tss was reduced in the Menin KO TH cells (Fig. 6e). In addition, the binding of other HATs, such as CBP and p300, to the Bach2 Tss in the Menin KO TH cells was comparable to that observed in the WT TH cells (Supplementary Fig. 8e). Therefore, it was shown that the primary result of Menin deficiency is the inability to maintain active histone modification at the Bach2 locus after TCR stimulation.

Menin controls Bach2 expression in the senescent TH cells. To determine whether the expression of Menin and/or Bach2 is reduced in senescent CD4 T cells, we established a senescent TH (sTH) cell induction model (Supplementary Fig. 9a). The CD62Llow/CD27high and CD62Llow/CD27low populations were increased in the sTH culture in comparison with that observed in the primary effector CD4 T cells (pTH) culture (Supplementary Fig. 9b). The number of IL-6-, IL-10- and OPN-producing cells was also increased in the sTH cultures that observed in the pTH culture (Supplementary Fig. 9c). Therefore, we concluded that sTH have senescent CD4 T-cell-like characteristics.

The mRNA and protein expressions of Bach2 in the sTH cells were lower than those observed in the pTH cells (Fig. 7a,b). In sharp contrast, the expression of Menin in the sTH cells was comparable to that observed in the pTH cells (Fig. 7a,b). Next, we performed ChIP sequencing using an anti-Menin antibody to compare the binding levels of Menin at the Bach2 locus in the sTH and in pTH cells. As shown in Fig. 7c, Menin binding was detected around the Tss of the Bach2 gene, spread towards the 3′ sites in the pTH cells. The level of Menin binding was decreased in the sTH cells, and a reduction in the H3K4me3 and H3K27ac levels was also detected in the sTH cells (Fig. 7d). The decreased binding of Menin around the Tss at the Bach2 gene locus was confirmed using ChIP-qPCR. A reduction in the binding of RNA polymerase II (PAPRII), PCAF, MLL1, Rbbp5 and Wdr5, as well as an increase in the binding of Suz12 at the Bach2 was also detected in the sTH cells (Fig. 7e). Although the reduced expression of Bach2 mRNA (Fig. 7d) and the level of H3K27ac (Supplementary Fig. 9d) in the sTH cells were
completely restored by TSA treatment (Fig. 7f), the recruitment of Menin at the Bach2 locus was not increased in the TSA-treated sTH cells (Supplementary Fig. 9e). These results indicate that the Menin-dependent maintenance of histone acetylation is required for the proper expression of Bach2, whereas Menin is not essential for Bach2 transcription.

Finally, we compared the expression levels of Bach2 in TH cells obtained from young and elderly mice. As shown in Fig. 7g, the Bach2 expression was lower in the elderly TH cells than in the young TH cells. In addition, a substantial number of elderly TH cells rapidly expressed an SA-β-Gal activity (Supplementary Fig. 10). These results demonstrate a correlation between ageing and the Bach2 expression.

**Discussion**

In this study, we demonstrated the critical role that the Menin-Bach2 pathway plays in regulating CD4 T-cell senescence and cytokine production. Menin KO TH cells exhibited phenotypes that are characteristics of cellular senescence, including a reduced growth rate, increased SA-β-Gal activity, prolonged activation of p65/RelA and induction of SASP after TCR stimulation. Furthermore, the generation of IL-4- and IFN-γ-producing cells was augmented in the Menin KO naive CD4 T cells in particular under IL-2 culture conditions. The transcription repressor Bach2, which inhibits senescence and abnormalities in cytokine production, was identified to be a downstream target of Menin. The transcription of Bach2 into the Menin KO TH cells restored the SAPS and cytokine production. In contrast, the Bach2 KO CD4 T cells rapidly expressed a senescent phenotype following TCR stimulation, with an increase in the generation of IL-4- and IFN-γ-producing cells in particular under IL-2 culture conditions. The decreased binding of Menin around Tss at the Bach2 gene locus in the pTH and sTH cells were determined using ChIP sequencing. The global patterns of histones H3K4me3 and H3K27ac at the Bach2 gene locus in the pTH and sTH cells were determined using ChIP sequencing and RNA polymerase II, KMT2A/2B components and Suz12 in the pTH and sTH cells.

Although the Th1 and Th2 cytokine production in young TH cells were not significantly different from those in elderly TH cells, a marked increase in the Th1 cytokine production was observed in the young TH cells. The Th1 cytokine production was completely restored by TSA treatment (Fig. 7f), the recruitment of Menin at the Bach2 locus was not increased in the TSA-treated sTH cells (Supplementary Fig. 9e). These results indicate that the Menin-dependent maintenance of histone acetylation is required for the proper expression of Bach2, whereas Menin is not essential for Bach2 transcription.

Finally, we compared the expression levels of Bach2 in TH cells obtained from young and elderly mice. As shown in Fig. 7g, the Bach2 expression was lower in the elderly TH cells than in the young TH cells. In addition, a substantial number of elderly TH cells rapidly expressed an SA-β-Gal activity (Supplementary Fig. 10). These results demonstrate a correlation between ageing and the Bach2 expression.

Discussion

In this study, we demonstrated the critical role that the Menin-Bach2 pathway plays in regulating CD4 T-cell senescence and cytokine production. Menin KO TH cells exhibited phenotypes that are characteristics of cellular senescence, including a reduced growth rate, increased SA-β-Gal activity, prolonged activation of p65/RelA and induction of SASP after TCR stimulation. Furthermore, the generation of IL-4- and IFN-γ-producing cells was augmented in the Menin KO naive CD4 T cells in particular under IL-2 culture conditions. The transcription repressor Bach2, which inhibits senescence and abnormalities in cytokine production, was identified to be a downstream target of Menin. The transcription of Bach2 into the Menin KO TH cells restored the SAPS and cytokine production. In contrast, the Bach2 KO CD4 T cells rapidly expressed a senescent phenotype following TCR stimulation, with an increase in the generation of IL-4- and IFN-γ-producing cells in particular under IL-2 culture conditions. The decreased binding of Menin around Tss at the Bach2 gene locus in the pTH and sTH cells were determined using ChIP sequencing. The global patterns of histones H3K4me3 and H3K27ac at the Bach2 gene locus in the pTH and sTH cells were determined using ChIP sequencing and RNA polymerase II, KMT2A/2B components and Suz12 in the pTH and sTH cells.
Menin autonomous reduction in the generation of myeloid, B and T cells TG mice, and a mixed bone marrow transfer assay revealed a cell-TSA treatment restored the H3K27 acetylation. This was further indicated by the finding that small intestinal inflammation occurs in Menin-MLL histone methyltransferase complex contains other transferases MLL1 (ref. 17) to regulate transcription. The Menin KO TH cells using immunoblotting, whereas a Menin interacts with histone methyltransferases MLL1 (ref. 17) to regulate transcription. The Menin-MLL histone methyltransferase complex contains other components encoded by Ash2l, Rbbp5 and Wdr5; however, the molecules do not always co-localize with Menin50. Moreover, it has been reported that Menin and MLL1 regulate distinct pathways during normal haematopoiesis and B-cell development51. We herein demonstrated that the primary effect of Menin deficiency was to reduce the H3K27ac, but not H3K4me3, levels at the Bach2 locus. The binding of the histone acetyltransferase, PCAF, at the Bach2 locus was decreased in the Menin KO TH cells. These results suggest that Menin forms complexes with PCAF that have not yet been identified and preserves the Bach2 expression in CD4 T cells by maintaining H3K27 acetylation. This was further indicated by the finding that TSA treatment restored the Bach2 expression in the Menin KO TH cells. In contrast, we were unable to detect Bach2 proteins in the Menin KO TH cells using immunoblotting, whereas a considerable amount of Bach2 mRNA continue to be expressed. Therefore, Menin may regulate the Bach2 expression via both transcriptional and post-transcriptional mechanism, thereby controlling CD4 T senescence and cytokine homeostasis during CD4 T-cell activation.

A recent report indicated that Menin has modest effects on haematopoiesis under steady-state conditions, while playing a crucial role in the long-term maintenance of haematopoietic stem cells (HSCs), particularly under conditions of haematopoietic stress52. A significant decrease in the number of B cells was observed in the tamoxifen-administered Menin<sup>Boxlox/fox</sup> x CreER<sup>Tg</sup> mouse, and a mixed bone marrow transfer assay revealed a cellular-autonomous reduction in the generation of myeloid, B and T cells from Menin-deleted HSCs52. We found that the number of mature T cells was modestly decreased in the spleens of the Menin<sup>Boxlox/fox</sup> x CD4-Cre TG mice. In addition, the number of Menin-deficient CD4 T cells further decreased after TCR stimulation. The present study indicates that Menin maintains the number of CD4 T cells in part by inhibiting cellular senescence. It is possible that Menin also plays a crucial role in inhibiting the senescence of HSCs induced by haematopoietic stress and is therefore required for the long-term maintenance of HSCs. Menin regulates haematopoiesis in part by regulating the Hox gene expression53. However, we were unable to detect a reduced Hox gene expression in the Menin KO TH cells using a DNA microarray analysis. Therefore, Menin presumably regulates CD4 T-cell senescence and cytokine production in a Hox-independent manner.

We detected prolonged activation of NF-κB p65 (RelA) in the Menin KO TH cells. The low level and prolonged activation of RelA is a common characteristic of senescent cells31,32, and RelA accumulates on the chromatin of senescent cells and acts as a master regulator of the SASP39. Menin interacts with the NF-κB components both in vitro and in vivo, and represses the transcriptional activation of an NF-κB reporter gene stimulated by phorbol-12-myristate-13-acetate or TNF-α27. Moreover, prolonged activation of RelA was also detected in the Bach2 KO TH cells. Therefore, Menin modulates the NF-κB-dependent signalling pathway in CD4 T cells via multiple pathways, thereby regulating the NF-κB-mediated induction of the age-dependent gene expression and senescence.

In conclusion, we herein demonstrated that Menin controls cellular senescence and cytokine production, and it is involved in the fate decision of CD4 T cells after TCR-mediated activation. Bach2 acts downstream of Menin in the regulation of the senescence and cytokine production. These findings reveal that Menin controls CD4 T-cell-mediated immune homeostasis, at least in part, by regulating the expression of Bach2. Moreover, Bach1, a homologue of Bach2, repressed the oxidative stress-induced cellular senescence of MEI23. Taken together, the data presented here indicate that Bach repressors may play a crucial role in repressing cellular senescence and in the subsequent age-associated development of various disorders, such as cancer and inflammatory diseases.

**Methods**

**Mice.** Mice, Menin<sup>Boxlox</sup> x Cre<sup>Tg</sup> KO mice and Cre<sup>Tg</sup> KO mice under the control of the Cd4 promoter were purchased from The Jackson Laboratory. Menin<sup>Boxlox</sup> x CD4 Cre<sup>Tg</sup> mice with BALB/c mice were generated by Dr Toshinori Nakayama (Chiba University, Chiba, Japan). Bach2<sup>Boxlox</sup> mice were established by Dr Tomohiro Kurosaki (RIKEN and Osaka University)26, OVA-specific 9 TCR (DO11.10) TH cells were provided by Dr D. Loh (Washington University School of Medicine, St Louis, USA)40, and C57BL/6 and BALB/c mice were purchased from Clea (Clea Japan, Tokyo, Japan). The female mice were used in in vivo experiments. For in vivo experiments, both male and female mice were used. All mice were maintained under specific pathogen-free conditions and were then used at 6-10 weeks of age. All experiments using mice received approval from the Kazusa DNA Research Institute and Ehime University Administrative Panel for Animal Care. All animal care was conducted in accordance with the guidelines of the Kazusa DNA Research Institute and Ehime University.

**Antibodies.** Antibodies used for intracellular and cell-surface staining were as follows: anti-IL-4-phycoerythrin (PE) mAb (11B11; BD Biosciences), IFN-γ-FTTC conjugated mAb (XM161.2; BD Biosciences), IL-13-PE (eBio13A; eBioscience), IL-2-APC mAb (JES6-5H4; eBioscience), IL-6-PE (MP5-20F3; BioLegend), IL-6-PE (RM4-4A; BioLegend), IL-10-APC (JES5-16E3; BD Biosciences), IL-17A-PE (TC11-18H10.1; BioLegend), OPN-PE (5F1C80B; R&D Systems), Foxp3-Alexa Fluor 647 (15010; BioLegend) and NF-κB p65-Alexa Fluor 647 (cat#448; Cell Signaling Technology), CD27-PE (LG.7F9; eBioscience), CD62L-APC (MEL-14; BD Biosciences), CTLA-4 (CD152) - PE (UC10-489; eBioscience) and anti-TCR DO11.10-PE (KJ1-26; BioLegend). Antibodies for immunoblotting were as follows: anti-Menin pAb (cat#A300-105A; Bethyl Laboratories), anti-Bach2 pAb (cat#S219202101; Thermo Scientific), anti-NF-κB p65 mAb (cat#8224; Cell Signaling Technology), anti-Rb pAb (sc-226; Santa Cruz Biotechnology) and anti-α-tubulin mAb (cat#MS-581-P; NeoMarkers). Antibodies used for ChIP assay were as follows: anti-histone H3K4me3 pAb (cat#39159; Active Motif), anti-histone H3K27ac mAb (cat#39133; Active Motif), anti-histone H3K27me3 pAb (cat#39155; Active Motif), anti-Menin pAb (cat#A300-105A; Bethyl Laboratories), anti-S2B pAb (cat#S219202101; Thermo Scientific), anti-RNA polymerase II mAb (8W16/22G; Thermo Scientific), anti-PCAF mAb (cat#3378; Cell Signaling Technology), anti-MLL pAb (cat#A300-086A; Bethyl Laboratories), anti-Rbbp5 pAb (cat#A300-109A; Bethyl Laboratories) and anti-Suz12 mAb (cat#3373; Cell Signaling Technology). All antibodies were diluted and used according to the manufacturer’s protocol.

**CD4 T-cell stimulation and differentiation in vitro.** Naive CD4 T (CD4<sup>+</sup>Cd62L<sup>hi</sup>/Cd25<sup>+</sup>/<sup>b</sup>_neg) T<sub>naive</sub> T cells were prepared by using a CD4<sup>+</sup>Cd62L<sup>+</sup> T-cell isolation kit II (Miltenyi Biotec). Naive CD4 T cells (1.5 × 10<sup>5</sup>) were stimulated with immobilized anti-TCR-β mAb (3 μg/ml ^{-1}, H57-597, BioLegend)}
and an anti-CD28 mAb (1 µg ml⁻¹, 37.5, BioLegend) for 2 days under the conditions indicated. Next, cells were transferred to a new plate and cultured further in the presence of cytokines. Cytokine conditions were as follows: IL-2 (Tc1) conditions: IL-2 (2.5 ng ml⁻¹); neutral conditions: IL-2 (2.5 ng ml⁻¹), anti-IL-4 mAb (5 µg ml⁻¹, 11B11, BioLegend) and anti-IFN-γ mAb (5 µg ml⁻¹, R-6-6A2, BioLegend).

Intracellular staining of cytokines. The cells that were differentiated in vitro were stimulated with immobilized anti-TCR-β mAb (1 µg ml⁻¹, H57-597, BioLegend) for 6 h with monensin (2 µM), and intracellular staining was performed as described. Flow cytometric analysis (FACS) was performed using a FACS Calibur (BD Biosciences), and results were analysed using FlowJo software (Tree Star).

ELISA and MILLIPLEX assay. The cells were stimulated with immobilized anti-TCR-β mAb for 16 h. The amounts of cytokines and OPN in the supernatants were determined by ELISA, as described. The amounts of chemokines were determined using an MILLIPLEX assay kit according to the manufacturer's protocol (EMD-Millipore).

Quantitative reverse transcriptase polymerase chain reaction determined using an MILLIPLEX assay kit according to the manufacturer's protocol (EMD-Millipore).

DNA microarray analysis. The gene expression profiles of the Menin KO naive CD4 T cells cultured under IL-2-conditions were analysed using the Agilent Whole Mouse 44K Array. The raw data were subjected to log₂ transformation and normalized using the Subio Platform (Subio).

Pull-down assay. Naive CD4 T cells were cultured under Tc1 conditions for 7 days. Then, the cells were pelleted, resuspended in buffer C (20 mM HEPES (pH 7.9), 420 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 1 mM dithiothreitol, 0.1% Nonidet P-40, 1 mM Na₂VO₃, 1 mM NaF, 1 mM β-glycerophosphate, 1 mM phenylmethylsulfonyl fluoride) and lysed on ice for 15 min. Insoluble material was removed by centrifugation. The supernatant was diluted 1:3 with buffer D (buffer C without NaCl). The lysates were incubated with 10 µg of poly(dI-dC)-poly(dI-dC) (#11-219-847, Roche) and streptavidin-agarose (#16-126, MILLIPIRE) carrying indicated biotinylated oligonucleotides for 40 min at 4°C. The bound proteins were eluted by adding SDS sample buffer and separated on a sodium dodeyl sulphate polyacrylamide gel and then subjected to immunoblotting with specific antibodies. Full size blots can be found in Supplementary Fig. 11. The NF-κB consensus oligonucleotide probes for the pull-down assay G-ATGGGAGGCACCTTCCCA GGC-3’. Renovirus-mediated gene transfer. Retrovirus infection was performed using pMXs-ires-hnGFR vector and a Plat-E packaging system, which is kindly, provided by Dr Toshio Kitamura (University of Tokyo, Japan). pMX-Bach2-ires-hnGFR was constructed by inserting mouse Bach2 cDNA into the multi-cloning site of pMXs-ires-hnGFR. Virus supernatant was concentrated by centrifugation for 6 h with monensin (2 µM), and intracellular staining was performed as described. Flow cytometric analysis (FACS) was performed using a FACS Calibur (BD Biosciences), and results were analysed using FlowJo software (Tree Star).

ChIP assay and ChIP-seq. The Magna ChIP Kit was used according to the manufacturer’s protocol (EMD-Millipore). Samples for ChIP-seq were prepared according to the manufacturer’s protocol (Illumina) and were sequenced using a Genome Analyzer Ix (Illumina). The obtained reads were mapped on the mouse reference genome sequence (9 nm) using the Bowtie 0.12.7 software program. Peak calling was performed using the MACS 1.0 software program. To visualize the ChIP sequencing results, the data were converted to a wiggle file format at a 25-base resolution and uploaded to the Subio platform (Subio, Japan).

Adoptive transfer. For in vivo expansion assay of the antigen-specific CD4 T cells, CD4 T cells from DO11.10 TG mice were sorted using an AutoMACS and transplanted into BALB/c mice. Then the mice were immunized i.p. with 100 µg OVA plus lipopolysaccharide (10 µg). OVA-TCR-β cells were detected using the KJ1-26 mAb. To assess the survival of Menin KO Tc1 cells in vivo, the CD45.1 WT or CD45.2 Menin KO naive CD4 T cells were cultured under Tc1 (IL-2-2) conditions. The WT (CD45.1) and Menin KO (CD45.2) effecter CD4 T cells were mixed 1:1 (5 × 10⁶ cells each) and transferred into the TCR-β0 KO mice. The ratio of CD45.1 to CD45.2 CD4 T cells was determined using FACS, at 4 weeks after transfer.

OVA-induced allergic airway inflammation. The WT and Menin KO mice were infected i.p. with 100 µg OVA in 2 mg of aluminium hydroxide gel (day 0) and challenged with OVA in saline intranasally (100 µg/mouse) on days 8 and 10. Two days later, BAL-fluid cells and lung samples were prepared for histological examination (H&E staining).

Infection with Listeria monocytogenes. WT and Menin KO mice were infected with Listeria monocytogenes (LM) (EY9 strain: 1 × 10⁵ CFU) intraperitoneally (primary infection), then reinfected with LM (5 × 10⁶ CFU) 14 days after the primary infection (secondary infection). The bacterial burden in the spleen was determined 3 days after each episode of infection.

Generation of senescent effecter CD4 T cells. KJ1-26 naïve CD4 T cells obtained from DO11.10 OVA-specific TCR TG mice were stimulated with plate bound anti-TCR-β mAb plus anti-CD28 mAb in the presence of IL-2 for 2 days, after which the cells were further expanded with IL-2 for an additional 3 days in vitro. The cells were then transferred intravenously into syngeneic BALB/c nu/nu recipient mice. Four weeks after the transfer, KJ1-26 transferred CD4 T cells were recovered from the spleens of the recipient mice and expanded using the same method as in the primary stimulation and used as a senescent effecter CD4 T (Vt4) cell.

Statistical analysis. Student’s t-test was used for the statistical analyses.

References
1. Linton, P. J. & Dohrn, K. Age-related changes in lymphocyte development and function. Nat. Immunol. 5, 133–139 (2004).
2. Finkel, T., Serrano, M. & Blasco, M. A. The common biology of cancer and aging. Nature 448, 767–774 (2007).
3. Gavazzi, G. & Krause, K. H. Ageing and infection. Lancet. Infect. Dis. 2, 659–666 (2002).
4. Lindstrom, T. M. & Robinson, W. H. Rheumatoid arthritis: a role for immunosenescence? J. Am. Geriatr. Soc. 58, 1565–1575 (2010).
5. Cavanaugh, M. M., Weyand, C. M. & Goronzy, J. J. Chronic inflammation and aging: DNA damage tips the balance. Curr. Opin. Immunol. 24, 488–493 (2012).
6. Maue, A. C. et al. T-cell immunosenescence: lessons learned from mouse models of aging. Trends Immunol. 30, 301–305 (2009).
7. Haynes, L. & Lefebvre, J. S. Age-related deficiencies in antigen-specific CD4 T cell responses: lessons from mouse models. Aging Dis. 2, 374–381 (2011).
8. Kuijlman, T., Michaloglou, C., Mooi, W. J. & Peeples, D. S. The essence of senescence. Genes Dev. 24, 2463–2479 (2010).
9. Adams, P. D. Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. Mol. Cell. 36, 2–14 (2009).
10. Rodier, F. & Campisi, J. Four faces of cellular senescence. J. Cell Biol. 192, 547–556 (2011).
11. Tchkonia, T., Zhu, Y., van Deursen, J., Campisi, J. & Kirkland, J. L. Cellular senescence and the senescent secretory phenotype: therapeutic opportunities. J. Clin. Invest. 123, 966–972 (2013).
12. Davalos, A. R., Coppe, J. P., Campisi, J. & Desprez, P. Y. Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metastasis Rev. 29, 273–283 (2010).
13. Ohtani, N. & Hará, E. Roles and mechanisms of cellular senescence in regulation of tissue homeostasis. Cancer Sci. 104, 525–530 (2013).
14. Franceschi, C. et al. Inflamm-aging: An evolutionary perspective on immunosenescence. Ann. NY Acad. Sci. 908, 244–254 (2000).
15. Brandi, M. L. et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J. Clin. Endocrinol. Metab. 86, 5658–5671 (2001).
16. Balogh, K., Patocs, A., Hunyadi, L. & Racz, K. Menin dynamics and functional insights take your partners. Mol. Cell Endocrinol. 326, 80–84 (2010).
17. Yokoyama, A. et al. Leukemia proto-oncoprotein MLL forms a SET1-like histone methyltransferase complex with menin to regulate Hox gene expression. Mol. Cell Biol. 24, 5639–5649 (2004).
18. Yokoyama, A. et al. The menin tumour suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. Cell 123, 207–218 (2005).
19. Chen, Y. X. et al. The tumor suppressor menin regulates hematopoiesis and myeloid transformation by influencing Hox gene expression. Proc. Natl Acad. Sci. USA 103, 1018–1023 (2006).
Schuettengruber, A., Martinez, A. M., Iovino, N. & Cavalli, G. Trithorax group proteins: switching genes on and keeping them active. Nat. Rev. Mol. Cell. Biol. 12, 799–814 (2011).

Hughes, C. M. et al. Menin associates with a trithorax family histone methyltransferase complex and with the hoxc8 locus. Mol. Cell. 13, 587–597 (2004).

Gao, S. B. et al. Suppression of lung adenocarcinoma through menin and polycomb gene-mediated repression of growth factor pleiotrophin. Oncogene 28, 4095–4101 (2009).

Balogh, K., Racz, K., Patocs, A. & Hunyady, L. Menin and its interacting proteins: elucidation of menin function. Trends Endocrinol. Metab. 17, 357–364 (2006).

Wu, T. & Hua, X. Menin represses tumorigenesis via repressing cell proliferation. Am. J. Cancer Res. 1, 726–739 (2011).

Sykiotis, G. P. & Bohmann, D. Stress-activated cap’n’collar transcription factors for memory CD8(+) T cell responses of low antigen affinity to protect against viral infection. Nat. Immunol. 10, 136–147 (2009).

Kometani, K. et al. Repression of the transcription factor Bach2 contributes to predisposition of IgG1 memory B cells toward plasma cell differentiation. Immunity 39, 136–147 (2013).

Roychoudhuri, R. et al. Bach2 represses effector programs to stabilize T-mediated immune homeostasis. Nature 498, 506–510 (2013).

Tsuchiya, S. et al. Bach2 maintains T cells in a naive state by suppressing effector memory-related genes. Proc. Natl Acad. Sci. USA 110, 10733–10740 (2013).

Dubois, P. C. et al. Multiple common variants for celiac disease influencing immune gene expression. Nat. Genet. 42, 295–302 (2010).

Cooper, J. D. et al. Meta-analysis of genome-wide association study data identifies novel type 1 diabetes risk loci. Nat. Genet. 40, 1399–1404 (2008).

Christodoulou, K. et al. Next generation exome sequencing of paediatric inflammatory bowel disease patients identifies rare and novel variants in candidate genes. Gut 62, 977–984 (2013).

Hu, G. & Chen, J. A genome-wide regulatory network identifies key transcription factors for memory CD8(+) T-cell development. Nat. Commun. 4, 2830 (2013).

Ondera, A. et al. STAT6-mediated displacement of polycomb by trithorax complex establishes long-term maintenance of GATA3 expression in T helper type 2 cells. J. Exp. Med. 207, 2493–2506 (2010).

Tlsty, J. S. et al. NF-kappaB inhibition delays DNA damage-induced senescence and lung adenocarcinoma during cellular senescence: causes and consequences. J. Exp. Med. 207, 2601–2612 (2012).

Adler, A. S. et al. Motif module map reveals enforcement of aging by continual NF-kappaB activity. Genes Dev. 21, 3244–3257 (2007).

Orsoro, F. G. et al. Nuclear lamina defects cause ATM-dependent NF-kappaB activation and linked accelerated aging to a systemic inflammatory response. Genes Dev. 26, 2311–2324 (2012).

Chien, Y. et al. Control of the senescence-associated secretory phenotype by NF-kappaB promotes senescence and enhances chemosensitivity. Genes Dev. 25, 2125–2136 (2011).

van Gisbergen, K. P. et al. The costimulatory molecule CD27 maintains clonally diverse CD8(+) T cell responses of low antigen affinity to protect against viral variants. Immunity 35, 97–108 (2011).

Fearon, D. T. The expansion and maintenance of antigen-selected CD8(+) T cell clones. Adv. Immunol. 96, 103–139 (2007).

Moro-Garcia, M. A., Alonso-Arias, R. & Lopez-Larrea, C. Molecular mechanisms involved in the aging of the T-cell immune response. Curr. Genomics 13, 589–602 (2012).

Shimataki, K., Nakashima, Y., Hattori, M., Hamazaki, Y. & Minato, N. PD-1 + memory phenotype CD4 + T cells expressing C/EBPalpha underlie T cell immunedeficiency in senescence and leukemia. Proc. Natl Acad. Sci. USA 106, 15807–15812 (2009).

Hendel, A., Hiebert, P. R., Boivin, W. A., Williams, S. J. & Granville, D. J. Granzymes in age-related cardiovascular and pulmonary diseases. Cell. Death Differ. 17, 596–606 (2010).