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Transcription Factors in Lymphocyte Development—T and B Cells Get Together

Meeting Review

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Immunologists who study lymphocyte development tend to identify themselves with either the "T" or "B" lymphocyte. In recent years, however, it has become clear that the transcription factors that control lineage specification are much more promiscuous than those who study them. Researchers working on transcription factors in both T and B lymphocyte development gathered together for what is probably the first time to discuss recent developments in the field (Madrid, October 19–21, 1998). The meeting was organized by J. M. Redondo (Centro de Biologia Molecular "Severo Ochoa," Madrid), S. Pettersson (Karolinska Institute, Stockholm) and P. Matthias (Friedrich Miescher Institute, Basel) and held at the Juan March Foundation in Madrid, Spain.

Specification of the B Lineage

In the past five years, enormous progress has been made in identifying transcription factors involved in the specification of the B lineage (reviewed by Reya and Grosschedl, 1998). Gene targeting experiments have revealed a set of five transcription factors, PU.1, Ikaros, E2A, EBF, and Pax5, that are essential for the generation or differentiation of B cell progenitors. Of these five regulatory proteins, the activity or expression of three, E2A, EBF, and Pax5, is specifically regulated within the B lineage of the hematopoietic system. Presentations at the meeting on these transcription factors provided new insight into the hierarchical ordering of their developmental functions and their roles in inducing or enabling lineage specification. Furthermore, experiments involving distinct approaches with the factors PU.1, E2A, and Pax5 provided new evidence for a close developmental relationship between the lymphoid and myeloid lineages, particularly between B cells and macrophages (Figure 1). Thus, this unique combination of five regulators may be both necessary and sufficient to induce the generation of B cell progenitors from a multipotential lymphoid–myeloid progenitor.

Specification of B lineage cells appears to be induced by the interaction of lymphoid or lymphoid–myeloid progenitors with stromal cells in hematopoietic tissue in conjunction with IL-7 signaling. As a consequence of these interactions, B cell progenitors expressing the cell surface proteins B220 and CD19 are generated (reviewed by Melchers et al., 1995). These proteins serve as early B lineage developmental markers and execute important functions in mature B cells. An early program of gene expression induced specifically in pro B cells also includes the genes encoding the immunoglobulin (Ig) receptor-associated signaling proteins Igα (mb-1) and Igβ (B29), and the surrogate light chains λ5 and VpreB. Regulated expression of the recombinase components Rag1 and Rag2 promotes rearrangement of V, D, and J gene segments at the Ig heavy chain (IgH) locus, which also appears to require developmentally programmed chromatin alterations associated with transcription of constant and variable germline (unrearranged) gene segments.

The hematopoietic transcription factor PU.1 is an Ets family member that is uniquely required for the generation of the lymphoid–myeloid system. It functions in a cell-autonomous manner to control the differentiation of multipotential lymphoid–myeloid progenitors (Scott et al., 1997). The PU.1 mutation results in a reduction of multipotential lymphoid–myeloid progenitors (AA4.1^{+} Lin^{-}) in the fetal liver, and these mutant cells fail to generate B cells or macrophages in vitro. These results support the view that the lymphoid and myeloid lineages develop from a common hematopoietic progenitor not shared with erythrocytes and megakaryocytes (Singh, 1996) (Figure 1). PU.1 controls macrophage development by regulating both proliferation and differentiation of macrophage progenitors (DeKoter et al., 1998). It regulates the transcription of the *M-CSF* receptor (c-*fms*) gene and therefore the responsiveness of macrophage progenitors to M-CSF. H. Singh presented data that suggested analogous functions for PU.1 in B cell development. *PU.1*$^{-/-}$ hematopoietic progenitors fail to express the IL-7 receptor because of an inability to induce the expression of the IL-7Rα gene. Such progenitors also do not express transcripts for the B lineage regulators EBF and Pax5. Retroviral transduction of *PU.1* cDNA into mutant progenitors induces IL-7 responsiveness and the generation of B220^{+}CD19^{+} B cell progenitors expressing the genes mb-1, B29, λ5, and VpreB. Thus, the block to B cell development caused by the *PU.1* mutation may be due to the combined defects in IL-7 signaling as well as the failure to induce expression of EBF and Pax5.

Signaling by IL-7R has been shown to regulate both proliferation and differentiation of B cell progenitors (Corcoran et al., 1998). Intriguingly, this study suggested that Pax5 expression in pro–B cells may be regulated by IL-7 signaling. Thus, PU.1 may indirectly regulate Pax5 expression by controlling IL-7 responsiveness. Failed expression of the IL-7R may partly account for the absence of T cell progenitors in *PU.1*$^{-/-}$ embryos. IL-7 is required for both B and T cell development and IL-7R is expressed on a common lymphoid progenitor (Kondo et al., 1997). It should be noted that expression of IL-7R in developing T cells is likely regulated in a PU.1-independent manner since expression of the *PU.1* gene is virtually undetectable in the thymus.

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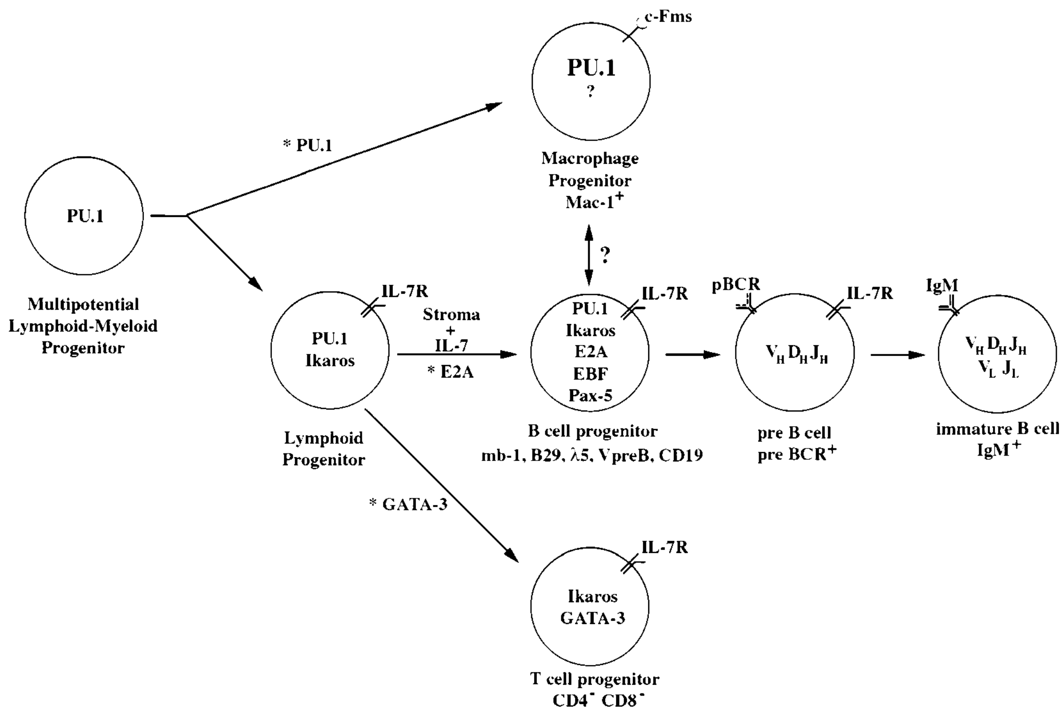

Figure 1. A Regulatory Model for B Cell Development

The *E2A* gene encodes two basic helix-loop-helix (bHLH) proteins, E12 and E47, which are generated by alternative splicing. Both proteins act in concert to promote B cell development (Bain et al., 1997). Very few $B220^+$ B cell progenitors are generated in $E2A^{-/-}$ mice. Such cells are blocked for Ig heavy chain gene rearrangement and fail to express Rag1, mb-1, λ5, and CD19 transcripts. C. Murre described ectopic, gain-of-function experiments with the *E12* gene product that suggested that *E2A* regulates EBF expression (Kee and Murre, 1998). He showed that conversion of the pre–B cell line 70Z/3 into a macrophage-like cell was accompanied by the loss of E2A and EBF activity. Ectopic expression of E12 in the macrophage-like cell induced B cell differentiation, including the expression of the *EBF, IL-7Rα, λ5,* and *Rag1* genes. Interestingly, ectopic expression of EBF resulted in the induction of a subset of E12-regulated genes. These experiments reinforce the developmental linkage between B lymphocytes and macrophages and suggest that the two differentiation programs may be interconvertible by manipulating key transcription factors (Figure 1). These results in conjunction with analysis of EBF function by the Grosschedl laboratory (Lin and Grosschedl, 1995; Sigvardsson et al., 1997), suggest that E2A functions both upstream of EBF by regulating its expression and also in concert with EBF to induce B cell–specific gene expression (for example, transcription of the surrogate light chain genes *VpreB* and *λ5*).

The *Pax5* gene encoding the transcription factor BSAP appears to function later than PU.1, Ikaros, E2A, and EBF in B cell development (Nutt et al., 1997; Thevenin et al., 1998). In Pax5 mutant bone marrow, $B220^+$ B cell progenitors are generated but cannot differentiate into pre–B cells. These mutant cells can proliferate in vitro in the presence of stromal cells and IL-7. They express wild-type levels of PU.1, Ikaros, E2A, and EBF transcripts. Furthermore, expression of *Rag1, Rag2,* and the surrogate light chain genes is unimpaired. However, Pax5 mutant pro–B cells express low levels of mb-1 and do not induce CD19 expression. Pax5 directly regulates transcription of these two B cell–specific genes (Nutt et al., 1998). Finally, Pax5 mutant pro–B cells are selectively impaired for V to DJ recombination at the IgH locus, a phenotype that is also associated with the IL-7R mutation (Corcoran et al., 1998). Thus, as noted above, IL-7R signaling may regulate Pax5 expression, which in turn would control V_H gene recombination by a novel mechanism.

M. Busslinger presented a series of collaborative experiments with T. Rolink and F. Melchers analyzing the developmental potential of Pax5 mutant pro–B cell clones. Intriguingly, in spite of their significant differentiation down the B cell pathway, these mutant cells were shown to be capable of giving rise to macrophages and osteoclasts in vitro as well as $CD4^+$ or $CD8^+$ T cells in vivo. It should be noted that pro–B cells arrested by the Rag2 mutation do not evidence such developmental plasticity in vitro. These results have two important developmental implications. First, they argue that Pax5 is necessary for commitment to the B lineage. In its absence, PU.1, Ikaros, E2A, and EBF generate B lineage progenitors that retain T lymphoid and macrophage developmental potentials. Second, the results of Busslinger and colleagues further strengthen the developmental connection between the lymphoid and myeloid lineages.

Figure 1 presents a regulatory model for B cell development. This model envisages that B cell differentiation is initiated by interaction of a progenitor cell expressing

PU.1, Ikaros, and IL-7R with stromal cells and IL-7. Stromal contact via an undefined signaling pathway may induce E2A activity (*E2A) in part by promoting the formation of B lineage–specific homodimers. Induction of E2A activity would then result in the expression of the downstream regulator EBF. In this model, interaction with stroma and signaling through IL-7R would also promote the expression of the remaining B lineage regulator Pax5. All of these transcription factors functioning in distinct combinations could induce the early program of B cell gene expression including the genes *mb-1, B29,* λ*5, VpreB, CD19, Rag1*, and *Rag2*. Similar scenarios based on the induction of a small set of key transcription factors can be proposed for the development of macrophage and T lineage progenitors. However, for these lineages fewer regulators have been described that function in specification. Induction of *PU.1* gene expression (*PU.1) may induce macrophage differentiation since PU.1 is expressed at significantly higher levels in the monocytic versus the B lineage (H. Singh). Finally, induction of GATA3 expression (*GATA3) may induce T cell differentiation (see below). It should be noted that in this model graded levels of PU.1 expression may participate in cell fate decisions (i.e., high levels favoring macrophage development; intermediate levels, B cell development; and its absence, T cell development).

The *Ikaros* gene encodes a family of zinc finger transcription factors that are essential for the generation of fetal B and T cell progenitors (Wang et al., 1996). *Ikaros* gene activity remains essential for adult B lymphopoiesis, but exhibits partial redundancy in postnatal T cell development. K. Georgopoulos reported that *Ikaros*$^{-/-}$ hematopoietic progenitors do not express transcripts for the FLK2 receptor, loss of which has previously been shown to cause a severe reduction in B cell progenitors. Although Ikaros proteins may function like PU.1 E2A, EBF, and Pax5 to positively regulate the early program of B cell–specific gene expression, a novel mechanism has been proposed whereby Ikaros complexes function as repressors in lineage specification (Brown et al., 1997). In nuclei of B lineage cells, Ikaros complexes have been colocalized with inactive T cell–specific genes, such as CD4 and CD8, and heterochromatin foci. An implication of these results is that Ikaros proteins may target genes expressed in developmentally related lineages to heterochromatic nuclear domains for inactivation. Thus, in the model outlined in Figure 1, Ikaros complexes may initially function to repress inappropriate myeloid gene expression in lymphoid progenitors and then participate in B and T cell specification by inactivating T and B cell–specific loci, respectively.

Transcription Factors Regulating B Cell Maturation and Activation

Proper differentiation of pro–B cells results in productive rearrangement of an Ig heavy chain allele ($V_HD_HJ_H$) and expression of the pre–B cell receptor (pre-BCR), composed of the μ heavy chain and the two surrogate light chains λ*5* and *VpreB*. Receptor feedback inhibits further rearrangements of heavy chain variable gene segments and promotes proliferation (Figure 1). During this phase surrogate light chain gene (λ*5* and *VpreB*) transcription appears to be repressed. Cessation of pre–B cell proliferation culminates in the induction of light chain gene (kappa or lambda) rearrangements and the generation of immature IgM$^+$ B cells. F. Melchers reviewed experiments using pro–B cell cultures from *Rag2*$^{-/-}$ mice which indicate that this later program of B cell–specific gene expression does not require signaling by the pre-BCR. Transcription factors regulating differentiation of pre–B cells remain to be genetically elucidated. Immature B cells that are not reactive to autoantigens are selected and mature into antigen-responsive, resting B cells (Figure 2). P. Matthias described experiments with OCA-B/OBF-1 mutant mice that suggested a role for this regulatory protein in B cell maturation. OCA-B is a B cell–specific coactivator that interacts with the POU domain transcription factors Oct1 and Oct2 (see below). Using a monoclonal antibody (MAb493) that distinguishes the developing B cell compartment in the bone marrow from peripheral mature B cells, P. Matthias in collaboration with T. Rolink and F. Melchers showed that OCA-B appears to be required for the exit of B cells from the bone marrow into the periphery. The splenic B cells that accumulate in *OCA-B*$^{-/-}$ mice appear to be generated during fetal development as they are eliminated in *OCA-B*$^{-/-}$ mice that are also deficient in the tyrosine kinase Btk, which is required for the generation of CD5$^+$ B cells.

Upon encountering cognate antigen, a naive B cell is stimulated to proliferate via signaling through its BCR, surface IgM (sIgM). The antigen is endocytosed, proteolytically processed, and presented to T helper (Th) cells. Extensive interactions of antigen-reactive B cells with cognate Th cells and follicular dendritic cells then result in the formation of germinal centers in secondary lymphoid tissue (reviewed by MacLennan, 1994). Clonal proliferation of B cells in germinal centers is accompanied by differentiation involving isotype switch recombination (ISR) and somatic hypermutation (SHM) of Ig genes (Figure 2). The process culminates in the generation of memory B cells or Ig-secreting plasma cells.

Transcription factors regulating B cell proliferation and differentiation can be divided into three functional groupings: (1) factors (e.g., Aiolos, PU.1, and Spi-B) that regulate initial signaling events by the BCR perhaps by controlling the expression of key signal transduction components, (2) factors that are activated upon signaling (e.g., NF-κB/Rel proteins that induce downstream gene expression), and (3) factors whose expression is induced upon B cell activation (e.g., OCA-B and Pip/IRF-4). K. Georgopoulos described the effect of the Aiolos mutation on B cell activation and differentiation. Aiolos encodes an Ikaros-related zinc finger transcription factor that is expressed at high levels in mature B cells. *Aiolos*$^{-/-}$ B cells hyperproliferate in response to BCR engagement and also exhibit a lower threshold for activation (Wang et al., 1998). Aiolos-deficient mice form germinal centers and have elevated serum IgG and IgE, in the absence of immunization. Interestingly, reduction of Ikaros activity in T cells results in a very similar cell cycle activation phenotype as that caused by loss of Aiolos in B cells. Thus, Aiolos and Ikaros appear to regulate the G0 to G1 transition as well as subsequent cell cycling. Their mechanisms of action remain to be elucidated.

Spi-B is an Ets family transcription factor that is highly related to PU.1. *Spi-B*$^{-/-}$ mice exhibit attenuated T-dependent immune responses (reduced serum IgG) that are

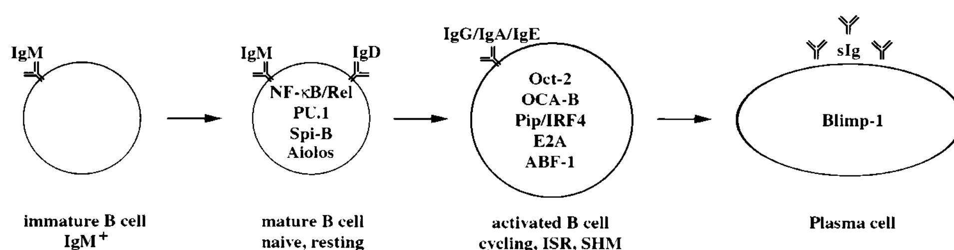

Figure 2. Transcription Factors Regulating B Cell Activation

associated with defects in germinal center formation and maintenance (Su et al., 1997). *Spi-B*$^{-/-}$ B cells are defective in BCR-stimulated proliferation. This defect is exacerbated in *Spi-B*$^{-/-}$, *PU.1*$^{+/-}$ B cells (C. Simon, personal communication). Molecular analysis of this defect suggests that Spi-B and PU.1 regulate expression of a novel membrane proximal BCR signaling component.

The B cell–specific coactivator OCA-B appears to regulate the activity of Ig gene promoters by associating with the POU domain transcription factors Oct1 or Oct2, which recognize the functionally essential octamer element. Mutation of the *OCA-B* gene does not result in a block to B cell development or diminished expression of IgM or IgD on mature B cells (Kim et al., 1996; Schubart et al., 1996). P. Matthias reported that B cells can be generated even in mice that lack both OCA-B and Oct2. These results suggest that the ubiquitous transcription activator Oct1 is sufficient to promote Ig gene transcription during the antigen-independent phase of B cell development. Although OCA-B is not essential for B cell development, it is required for immune responses against T-independent and T-dependent antigens. This appears to be caused by a failure to form germinal centers upon immunization. Given the cell-autonomous requirement for OCA-B function and the synergistic induction of its expression by signaling pathways that induce germinal center formation, it has been proposed that OCA-B is specifically required for the germinal center reaction (Qin et al., 1998). Key OCA-B target genes underlying such a function remain to be identified. P. Matthias proposed a molecular model for the Oct1/OCA-B/DNA complex that envisages OCA-B as a molecular clamp interacting with both subdomains of the bipartite POU domain (Sauter and Matthias, 1998). Site-directed mutagenesis localized residues L6 and E7 in the POUS domain and K155 and I159 in the POUH domain as being critical for ternary complex formation. Interestingly, OCA-B can promote transactivation by recruiting artificially separated POU domains to an octamer element in vivo.

Pip/IRF-4 is a lymphoid-restricted IRF transcription factor that is recruited to composite elements within Ig light chain enhancers through a specific interaction with PU.1 (Brass et al., 1996). Mice deficient in Pip/IRF-4 can generate normal numbers of B cells that express IgM with kappa or lambda light chains. However, these mice exhibit a dramatic reduction in serum Ig levels and do not mount detectable antibody responses to T-dependent as well as T-independent antigens (Mittrucker et al., 1997). Pip is recruited to its binding site on DNA by phosphorylated PU.1. H. Singh described a model for PU.1/Pip ternary complex formation. In this model, PU.1/Pip interaction is DNA template-directed and involves two distinct protein–protein interaction surfaces: (1) the Ets and IRF DNA-binding domains and (2) the phosphorylated PEST region of PU.1 and a lysine-requiring putative α helix in Pip. To analyze the function of these factors in vivo, the Singh laboratory engineered chimeric repressors containing the Ets and IRF DNA-binding domains connected by a flexible POU domain linker. When stably expressed, the fused dimer strongly repressed the transcription of a rearranged Igλ gene in a plasma cell line (Brass et al., 1998). Thus, Pip is dispensable for light chain gene transcription during B cell development but may be required for enhanced expression of these genes during B cell activation.

A third highly specific ternary complex important for B cell–specific gene expression was reported by J. Hagman. This complex involves Pax5, which recruits specific Ets family proteins (e.g., Ets1) to a composite element in the mb-1 gene promoter. In this case the complex requires only the Pax5 paired box and the Ets DNA binding domains (Fitzsimmons et al., 1996). By tethering deletion fragments of Pax5 to a heterologous DNA-binding domain, the Hagman laboratory has delineated a 73–amino acid segment that is sufficient for Ets1 recruitment. This segment contains a highly conserved β hairpin motif that also enables other Pax family members to interact with Ets factors (Wheat et al., 1999). Intriguingly, Pax5 appears to negatively interact with the Ets factor PU.1 to modulate IgH gene transcription in B cells. S. Pettersson reported that the IgH 3′ enhancer contains functionally important binding sites for Pax5 and PU.1 separated by 44 bp. Pax5 was shown to repress enhancer activity stimulated by PU.1. The mechanism underlying this antagonistic interaction between Pax5 and PU.1 remains to be elucidated.

C. Murre described the cloning and characterization of a novel bHLH transcription factor, ABF1, expressed in activated B lymphocytes. ABF1 can bind E box motifs in Ig gene enhancers either as a homodimer or as a heterodimer with E2A proteins (Massari et al., 1998). To analyze the function of E2A complexes during B cell activation, the Murre laboratory transiently expressed Id3, a negative regulator of E2A proteins, in activated B cells. Expression of Id3 appears to selectively inhibit Ig isotype switching. E2A complexes could potentially regulate isotype switching by binding to enhancer elements at the 3′ end of the IgH locus.

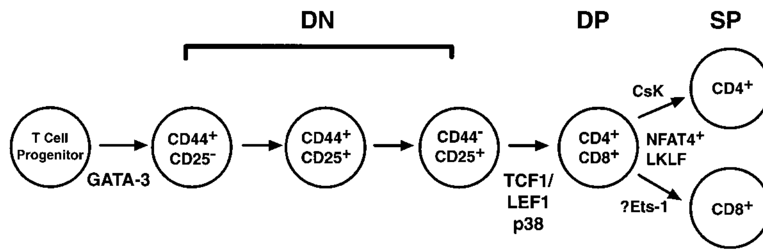

Figure 3. Transcription Factors that Regulate Development of the T Cell Lineage

Specification of the T Lineage

In the thymus, bone marrow–derived stem cells undergo a programmed series of differentiation and selection steps that results in the generation of mature functional $CD4^+$ and $CD8^+$ cells that are exported to the periphery (Figure 3). The earliest stage of thymocyte development is the so-called double-negative (DN) cell that lacks expression of both the CD3/TCR complex and the CD4 and CD8 coreceptors. DN cells then express the *Rag1* and *-2* genes, rearrange the *TCR*β gene ($CD3/TCR^+$) and express CD4 and CD8 coreceptors to become double-positive (DP) cells. DP cells rearrange the *TCR*α gene and undergo both positive and negative selection through interaction with major histocompatibility complex (MHC) molecules on thymic epithelium and dendritic cells to become SP $CD4^+$ helper or $CD8^+$ cytotoxic T cells. An ever-expanding array of transcription factors and signaling molecules plays distinct roles in each step of this selection process as schematized in Figure 3 and detailed at length in an excellent recent review (Kuo and Leiden, 1999). Here we will focus primarily on those transcription factors that uniquely affect the generation of T cells rather than those such as Ikaros, PU.1, and E2A (as described above), which target a common lymphoid–myeloid progenitor and ablate both T and B cell development.

The GATA3 zinc finger transcription factor is positioned at the very earliest stages of the T cell lineage. The differentiation of $GATA3^{-/-}$ ES cells is blocked at or before the earliest DN stage of thymocyte development as evidenced by the complete failure of $GATA3^{-/-}$ ES cells to contribute to the DN thymocyte population (Ting et al., 1996). As discussed below, GATA3 also influences Th2 differentiation, thus playing a role in multiple stages of T cell development. The identification of GATA3 target genes in the earliest committed DN thymocytes as well as in committed mature Th2 cells will be an important area of investigation. Lymphoid enhancer factor 1 (LEF1) and T cell factor 1 (TCF1) are closely related members of the "HMG box" family of transcription factors that regulate the TCRα enhancer in vitro. Disruption of either the *Tcf1* or *Lef1* genes results in an incomplete (*Tcf1*) or no (*Lef1*) defect in thymocyte development (Okamura et al., 1998). Thymocytes from older mice lacking *Tcf1* are arrested at the DN stage (H. Clevers) but, interestingly, T cells can be briefly generated (Verbeek et al., 1995). The nature of the defect is in both the maintenance and the generation of T cells, since $Tcf1^{-/-}$ mice have normal T cells at birth but, by 2 weeks of age, have arrested development in the thymus. There is some redundancy between *Tcf1* and *Lef1*, since double mutant mice have a complete arrest at the $CD8^+$ ISP stage (Okamura et al., 1998). Another HMG family member, *Sox4*, is also critical in the transition from the DN to the DP stage (van de Wetering et al., 1993). The presence of similar defects in *Tcf1/Lef1* and *Sox4* mutant mice suggest that each of these three proteins is required for optimal proliferation and maturation of DN thymocytes, and maintenance of the peripheral pool of mature T cells. How might this be accomplished? Although Tcf1/Lef1 regulate the expression of the *TCR*α gene, downregulation of TCRα cannot explain the block since TCRα expression is not required for the DN-to-DP transition. The answer may lie in the recent discovery, presented by H. Clevers, that TCF/LEF1 proteins are downstream targets of the Wnt/Wingless signaling pathway shown to control development in *Drosophila* and *Xenopus*. TCF/LEF proteins mediate Wnt/Wingless signaling by direct interaction with and recruitiment of Armadillo/β-catenin as a transcriptional coactivator (Behrens et al., 1996). However, Tcf factors can also act as repressors in *Drosophila, Xenopus*, and *Caenorhabditis elegans*. H. Clevers presented an explanation for this transcriptional repression by his demonstration that the *Xenopus* Wnt effector XTcf3 interacts with both Groucho-related transcriptional repressors and activators (Roose et al., 1998). The interaction of a single transcription factor with both activator and repressor proteins to achieve appropriate expression of target genes in cell-fate decisions is an area of intense investigation in developmental biology (Gray and Levine, 1998) and emerged as a theme of this meeting. The identification of activator- and repressor-controlled target genes for Tcf1 in lymphocytes will be an important area since such genes can be predicted to be critical in the maintenance of lymphoid stem cells.

Two signaling pathways in addition to Wnt appear to be critical as negative rather than positive regulators for the generation of T cells. The constitutive activation of the p38 MAP kinase pathway in the thymus blocks the differentiation of DN into DP thymocytes, suggesting that downregulation of p38 kinase activity is important for this transition, as presented by M. Rincón. Conditional ablation of the Csk kinase, which inhibits Src family kinases (Lck, Fyn) by phosphorylating C-terminal tyrosine residues, completely overcomes the DN-to-DP block and allows CD4 thymocyte development in both TCRβ-deficient and MHC class II–deficient mice (Schmedt et al., 1998). A. Tarakovsky speculated that the function of Csk is to make T cell development antigen dependent, via the regulation of the two known targets of Csk, Lck and Fyn, shown to be sufficient to control CD4 T cell development.

Interestingly, $CD8^+$ cells are not generated in the absence of Csk, providing support for earlier suggestions

that thymocyte commitment to the CD8 and CD4 lineages is asymmetric (Suzuki et al., 1995). CD8 commitment requires MHC-dependent signals while CD4 commitment is MHC-independent and may occur by default (Suzuki et al., 1995). The molecular basis for this asymmetry is unknown but may stem in part from differences in the transcription factors that control the expression of the coreceptor molecules as DP thymocytes traverse to the SP stage. An intronic silencer has been reported to control the tissue-specific expression of CD4 in T helper cells. It now appears from work presented by G. Siu that there are at least two factors binding to the silencer: HES1, a bHLH protein that is a homolog of the *Drosophila* Hairy/Enhancer of split (Kim and Siu, 1998), and a novel homeodomain protein, termed SAF, cloned by a yeast one-hybrid approach. Mutation of the HES1- and SAF-binding sites affects function, suggesting for HES1, that the Notch signaling pathway is important in thymic development (Kim and Siu, 1998). Interestingly, E. Robey has recently presented data that constitutive activation of Notch results in upregulation of *HES1* RNA. One can speculate that the transcription factor Ets1, critical in the devlopment of natural killer cells, may also be required to permit the full differentiation of CD8 cells since $CD8^{+}$ Ets1-deficient T cells express low levels of CD4 (Kuo and Leiden, 1999). J. Leiden hypothesizes that Ets1 may control the expression of important proximal signal transduction molecules whose dysregulation results in abnormal CD8 T cell development.

Csk may allow cells to adapt to signals of varying strength by altering threshholds of T cell activation (A. Tarakhovsky). An interesting theme that emerged from this meeting is that transcription factors also control signaling threshholds in lymphocytes, similar to the regulation of development in *Drosophila* and *C. elegans* by transcription factor gradients. K. Georgopoulos reported that disruption of the *Ikaros* gene in lymphocytes leads to a decreased number of peripheral T cells that both hyperproliferate and enter the cell cycle more rapidly than wild type, with a shortened G1 phase. A similar phenotype is observed in T cells from mice lacking both the NFATp and NFAT4 transcription factors (L. Glimcher), while lymphocytes lacking NFATc have the opposite phenotype (Ranger et al., 1998a; Yoshida et al., 1998). E. Serfling suggested (see below) that alternative polyadenylation of NFATc might be one mechanism to generate high threshhold levels after stimulation in T effector cells. The downstream target genes for these factors are largely unknown but may be involved in the maintenance of the resting state, in control of the cell cycle, or in the regulation of a T cell–specific death pathway. One interesting candidate is the gene encoding the LKLF transcription factor, recently shown by Leiden and colleagues to be critical both in controlling the DP-to-SP transition in the thymus and in maintaining T cell quiescence in the periphery (Kuo et al., 1997). The hyperactivation and increased rates of apoptosis of $LKLF^{-/-}$ T cells suggest that the maintenance of quiescence is not a passive process, but one actively regulated at the level of transcription.

At the DN stage, the T cell lineage splits to generate two distinct types of T cells, distinguished by the type of T cell receptor they bear—TCR$\alpha\beta$ and TCR$\gamma\delta$. Transcription factors that regulate the expression of each

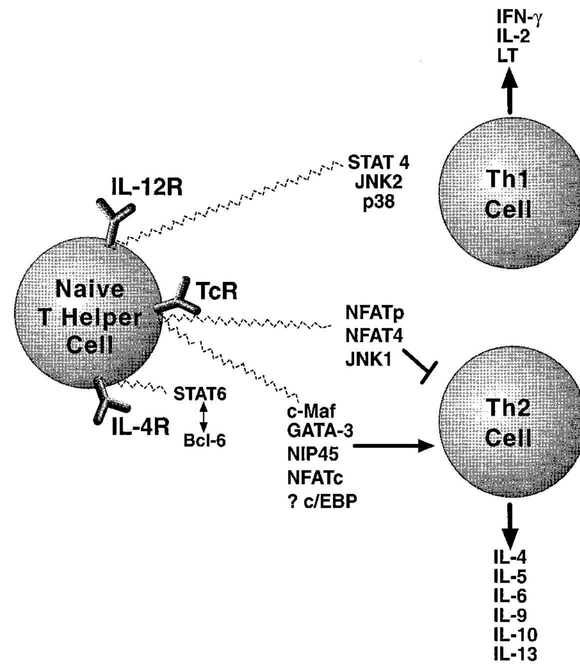

Figure 4. Transcription Factors that Control T Helper Lineage Commitment

TCR chain gene have been identified. Although the development of the $\gamma\delta$ lineage is distinct from $\alpha\beta$, data presented at this meeting suggest that there is reciprocal regulation between them evident at the level of transcription. Using in vivo footprinting, M. Krangel and colleague C. Hernandez-Munain demonstrated that the occupancy of the CBF, Myb, and GATA sites in the δ enhancer in DN cells decreases at the DP stage at the time when the Eα enhancer is active. This may serve as a mechanism to prevent previously rearranged (in frame) δ genes from being expressed in cells that are differentiating along the $\alpha\beta$ pathway. A related series of experiments emphasizes the value of looking at promoter occupancy in vivo to ascertain both accessibility of chromatin to factor binding and cooperative interactions between transcription factors. Thus, M. Krangel reported that sites in the Eα enhancer were occupied in DN cells of $Rag^{-/-}$ mice despite the absence of Eα transcripts, and that mutation of either the Tcf/Lef or Ets site in the Eα enhancer resulted in generalized failure of enhancer occupancy at all other sites (Hernandez-Munain et al., 1998).

Transcription Factors that Control T Helper Cell Development

Naive $CD4^{+}$ T helper precursor cells respond to antigen by differentiating along a Th1 (IFN-γ, IL-2) or Th2 (IL-4, -5, -6, -9, -10, -13) pathway (Figure 4). These two subsets of Th cells are defined by the patterns of cytokines they produce. This differentiation is highly dependent on the presence of certain cytokines and their downstream signaling transcription factors. Thus, Th1 and Th2 development do not occur in the absence of IL-12/Stat4 (Thierfelder et al., 1996; Kaplan et al., 1996b) and IL-4/Stat6 (Kaplan et al., 1996a), respectively. Using in vivo DNase I hypersensitivity assays to assess locus accessibility,

A. Rao reported rapid chromatin remodeling and CpG demethylation as demonstrated by the acquisition of tissue-specific hypersensitive sites, after antigen stimulation and polarization of both naive and mature effector T cells (Agarwal and Rao, 1998). Coordinate remodeling of the IL-4/IL-13/IL-5 locus on chromosome 11 and at the IFN-γ locus in Th2 and Th1 cells, respectively, was observed. She suggested a two-step model in which antigen-driven chromatin remodeling is followed by antigen- and cytokine-dependent occupancy of DNA by tissue-specific transcription factors.

Considerable progress has recently been made in identifying such tissue-specific factors for the development of the Th2 compartment. c-Maf is a Th2-specific basic region/leucine zipper protein that transactivates the IL-4 promoter in vitro (Ho et al., 1996; Hodge et al., 1996a). The provision of three factors, the Th2-specific c-Maf protooncogene, NFAT, and an NFAT-interacting protein, NIP45, has been previously shown to reconstitute endogenous IL-4 production in nonproducer cells (Hodge et al., 1996a). The phenotypes of c-Maf and NFAT genetic mutant mice recently produced provide compelling evidence that c-Maf and NFAT proteins control the Th2 differentiative program (Mach et al., 1994; Ho et al., 1998; Oukka et al., 1998; Ranger et al., 1998a, 1998b). Evidence for a critical role for c-Maf in driving IL-4 production in normal T cells was provided by studies reported by L. Glimcher demonstrating overproduction of Th2 cytokines in c-Maf overexpressor transgenic mice and severely impaired IL-4 production in mice lacking c-Maf. Since c-Maf-deficient T cells can produce small amounts of IL-4 when stimulated with exogeneous IL-4, it is likely that IL-4-responsive factors such as GATA3 (Zheng and Flavell, 1997) and Stat6 may also contribute to Th2 development. Indeed, mice lacking Stat6 do not develop Th2 cells (Kaplan et al., 1996a), while mice overexpressing GATA3 have an increased number of Th2 cells (Zheng and Flavell, 1997). However, unlike c-Maf, GATA3 probably does not directly regulate IL-4 transcription although it clearly does directly transactivate the IL-5 gene (Zhang et al., 1997; Ouyang et al., 1998). It may instead act further upstream, perhaps as a chromatin remodeling factor, to make the IL-4/IL-5 locus accessible. Another transcription factor, C/EBPβ, has been shown to specifically increase transcription of the human IL-4 gene (Li-Weber et al., 1997) although its expression is not Th2-limited in murine cells (Ho et al., 1996). In sum, while significant advances have been made in identifying a set of transcription factors important in developing the Th2 lineage, much remains to be learned about how this set of Th2-specific transcription factors are regulated in response to extracellular signals, as well as the nature of the combinatorial interactions that occur on Th2 cytokine promoters. Furthermore, there is some evidence that a balance between repressors and activators is required to maintain a Th1/Th2 balance. Thus, the transcription factor Bcl6 may compete with Stat6 to control the development of the Th2 lineage as demonstrated by the profound Th2 phenotype of mice lacking Bcl6 (Dent et al., 1997).

Three of the four known NFAT proteins are expressed in both Th1 and Th2 cells and can transactivate both Th1 and Th2 cytokine gene promoters in vitro (Rao et al., 1997). Nevertheless, the phenotype of mice deficient in either NFATp or NFATc suggested a Th-selective and reciprocal role for these proteins in regulating the balance of Th1/Th2 responses. Severe reductions in IL-4 production were noted in *NFATc*$^{-/-}$ lymphocytes (Ranger et al., 1998a; Yoshida et al., 1998), while enhanced IL-4 production was observed in *NFATp*$^{-/-}$ mice (Hodge et al., 1996b; Xanthoudakis et al., 1996). L. Glimcher reported that mice lacking both NFAT4 and NFATp had extreme and selective activation of the Th2 compartment accompanied by constitutive nuclear localization of NFATc, suggesting a repressor role for NFATp and NFAT4 and an activator role for NFATc in controlling Th2 development and activation (Ranger et al., 1998b). What is the mechanism by which different NFAT proteins selectively regulate distinct cytokines? There may be several levels of control, including transcriptional and posttranslational regulation of NFAT. NFATc for example is present in alternatively spliced isoforms that differ in naive and activated T cells (E. Serfling). A host of different kinases including GSK3, JNK, and CKIa, that regulate NFAT nuclear export have recently been described (Beals et al., 1997; Zhu et al., 1998), some of which phosphorylate only the NFAT4 protein (Chow et al., 1997). C. Dong from the Flavell laboratory reported that Th cells from JNK1-deficient mice were hyperresponsive to TCR stimulation and preferentially became Th2 effector cells (Dong et al., 1998). This phenotype was similar to that of mice lacking NFATp and also was accompanied by constitutive nuclear localization of NFATc as described by Glimcher (Ranger et al., 1998b). J. Redondo described yet another MAP kinase, p38, to be involved in the nuclear export of NFATp. It is probable that different signaling pathways will regulate distinct NFATs in a cell-type specific manner. It is also likely that NFAT proteins balance Th differentiation both by partnering with distinct coactivators and repressors and by inducing distinct sets of target genes. Known NFAT target genes include cytokines, fasL, and CD40L receptors, but there will surely be others. Two newly identified NFAT target genes were reported to be the IL-2 receptor α chain (CD25) (Schuh et al., 1998) and cyclooxygenase 2 (*Cox2*), shown to be an immediate early gene in T cells (M. Fresno). M. Fresno reported that *Cox2* is both regulated by NFAT and itself regulates NFAT activity. The identification of NFAT kinases that affect NFAT activity in vivo as well as the isolation of additional NFAT target genes will be critical in understanding how NFAT proteins balance the Th immune response.

Much less is known about factors that control Th1 development. Mice lacking IL-12 or its downstream signaling factor, Stat4, do not develop Th1 cells (Kaplan et al., 1996b; Thierfelder et al., 1996). Th1 cells are also lacking in mice deficient for the transcription factor IRF-1, likely through its direct effect in controlling transcription of the IL-12 gene (Lohoff et al., 1997; Taki et al., 1997). Activation of the p38 MAP kinase pathway by the expression of constitutively activated MAP kinase kinase 6 (MKK6) boosts IFN-γ production, while blockade of this pathway by expression of a dominant negative p38 MAP kinase or by targeted disruption of JNK2 partially impairs Th1 responses (Rincón et al., 1998;

Yang et al., 1998). Further, Th1 cell development is somewhat impaired in the absence of JNK1, consistent with its placement upstream of p38 and downstream of MEKK6 (Dong et al., 1998). The mechanism by which the JNK2 signaling pathway affects Th1/Th2 balance is unknown but might be via a direct effect on transcription of the IFN-γ gene. Possible downstream targets include the ATF-2 and AP-1 transcription factors, both substrates of Jnk kinases and also known to bind to sites in the IFN-γ promoter. However, we still do not know the identity of the transcription factors responsible for the tissue-specific expression of IFN-γ or other Th1 cytokines in Th1 cells. Unless the Th1 pathway is a default pathway, we should expect there to be factors equivalent to c-Maf and GATA3 that drive this lineage. Alternatively, transcription factors that act as activators in Th2 cells might have an equally important but opposing function as repressors in Th1 cells. In this regard it is intriguing that both c-Maf and GATA3 partially inhibit production of the Th1 cytokine IFN-γ (Ho et al., 1998; Ouyang et al., 1998). The relationship between signaling threshhold levels and Th1/Th2 balance is also intriguing. Lowering this threshhold appears to favor formation of Th2 cells as exemplified by the phenotype of mice lacking NFATp and NFAT4. It will be of interest to determine the role of Th1-specific transcription factors, when they are isolated, on T cell proliferative responses.

An interesting point to emerge from this meeting was that signaling proteins and/or transcription factors act at multiple stages of T cell differentiation. GATA3 is a critical regulator of both early T cell development and the transition of a mature Th precursor cell to the Th2 lineage, consistent with the presence of GATA3-binding sites in the promoters of many T cell–specific genes (Ting et al., 1996). Alteration of the Erk/Jnk pathway affects both thymocyte development and mature Th differentiation, while NFAT4 plays a role both in generation and survival of thymocytes and in determining the activation state of peripheral T lymphocytes (Oukka et al., 1998). Further, the demonstration that chromatin remodeling is involved in a very early stage of cytokine induction (Agarwal and Rao, 1998) implies that the NFAT, Stat, GATA3, and c-Maf transcription factors may also act at this step as well as at the step of acute induction of transcription. Transcription factors can simultaneously affect the development of the T and the B lineages. Thus, Ets1 is necessary to achieve a normal thymocyte number and is also required to prevent spontaneous differentiation of B cells into plasma cells (Kuo and Leiden, 1999). Clearly, it will be necessary to identify the interacting partners and patterns of gene expression induced by each of these transcription factors in the thymus versus the peripheral lymphoid compartment.

Transcription Factors that Control Lymphocyte Proliferation and Transformation

Many examples now exist of tumorigenesis arising from deregulation of transcription factor function consequent to chromosomal translocations. However, transcription factors may also promote or impede tumorigenesis by other mechanisms as exemplified by the anti-oncogenes or "tumor suppressors" p53 and Rb. We have mentioned earlier that certain of the factors discussed above negatively control signaling threshholds in lymphocytes, leading to hyperproliferation and in some cases transformation. In the "multistep" model of carcinogenesis, a single genetic alteration is insufficient to achieve transformation but requires other cooperating cell alterations. The dysregulation of cell growth observed in the absence of the transcription factors described above may serve as the "first hit" and also result in an increased tendency for a "second hit" to occur, leading to frank transformation. Several examples were provided at this meeting. A dominant-negative mutation in the *Ikaros* gene leads to rapid development of lymphoma and leukemia (Winandy et al., 1995), while Ikaros null mice also develop lymphomas albeit at a slower pace (K. Georgopoulos). H. Clevers reported that in colonic epithelium deficient for APC, constitutive activity of Tcf4, closely related to the lymphoid specific Tcf1, and also a downstream effector of Wingless/Wnt signaling, results in transformation while the absence of Tcf4 results in depletion of epithelial stem cell compartments (Korinek et al., 1998). Since absence of Tcf1 similarly results in the depletion of the lymphoid stem cell compartment, it could be predicted that constitutive expression of Tcf1 will lead to lymphoid tumors. C. Scheidereit reported that constitutive nuclear activity of the NF-κB p65 and p50 heterodimer is present in the Reed-Sternberg cells of Hodgkins disease and this constitutive activation is secondary either to loss of functional IκBα or to aberrant constitutive IκB kinase activation (Bargou et al., 1997; Krappmann et al., 1999). Blocking endogeneous NF-κB suppresses proliferation of these malignant cells. Are the IκB proteins and these other transcription factors in fact tumor suppressors, and if so, what is the mechanism by which they affect cellular proliferation? Current investigations focus on the role of these factors in interacting with the cell cycle machinery. Preliminary data was presented by C. Scheidereit that NF-κB activity is required for the G1/S transition in mouse embryonic fibroblasts via controlling the rate of pRb phosphorylation. NF-κB also directly transactivates the cyclin D promoter (M. Hinz et al., submitted). Uncovering the relationship between transcription and transformation for factors involved in T and B cell development will be a hotly investigated area for the future.

Conclusions

The B and T lineages of the lymphoid system represent leading models for analysis of developmental pathways in mammals. The Juan March meeting provided a unique forum for presentations on transcription factors that orchestrate development of these related lineages. The B and T lineages share two key developmental principles, namely (1) assembly of antigen receptor gene segments utilizing a common recombination apparatus and (2) use of rearranged Ig heavy chain or TCRβ genes to regulate key developmental transitions. It is interesting to note that of the various transcription factors required for lymphocyte development, the Ikaros and related family of proteins may perform shared molecular functions in regulating both the development and proliferation of B and T lineage cells. These functions would include repression of lineage-inappropriate genes and setting thresholds for lymphocyte activation. Considerable progress is being made in identifying and analyzing regulators that function to specify cell fate decisions at various nodal points in the lymphoid system, for example, during

the generation of (1) B versus T lineage progenitors, (2) CD4^{+} vs CD8^{+} single-positive T cells, and (3) Th1 versus Th2 helper T cells. In the latter two cases, signaling pathways involving Notch or cytokine receptors (IL-12R, IL-4R), respectively, appear to induce cell fate specification. Lineage specification in each instance seems to involve positive regulation of the lineage-appropriate gene set and active repression of lineage-inappropriate genes. Transcription factors function in a combinatorial capacity at two distinct levels: (1) in specifying lineage fates perhaps by establishing stable cross-regulatory networks and (2) in regulating lineage-specific structural genes by assembling specific multiprotein DNA complexes. Thresholds of signaling molecules and transcription factors likely regulate proliferation and differentiation of lymphocytes and their progenitors. Molecular analysis of such thresholds will be a key area for further investigation.

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