Control of gene expression during lymphoid development: targeted gene disruption provides new clues

Review article

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Most recent concepts of molecular control of cellular differentiation pathways are derived from genetic studies on the fruitfly Drosophila melanogaster. Cascades of regulatory genes, many of which encode transcription factors, control the organization of the body plan, also called pattern formation (21, 30). The transcriptional regulation of genes is thought to be mediated by the binding of transcription factors to cis-acting regulatory elements: promotors, silencers and enhancers. These transcription factors are generally modular proteins. Different domains within these proteins are involved in different protein functions, such as DNA-binding of transcriptional transactivation (2, 14, 20, 22, 26). Together, the complex network of interactions between transcription factors and the regulatory elements of structural genes is thought to be responsible for the control of cellular phenotype, and consequently of entry of cells into the right developmental pathway.

During lymphoid development, phenotypical changes that occur have been described in great detail. Together, these data make the lymphoid system probably the best characterized developmental system in higher eukaryotes. Although most phenotypical changes have been described in great detail, the underlying molecular processes remain poorly understood. For many higher eukaryotes, the classical genetic approach of screening large numbers of mutagenized animals is not feasible. For these organisms, a strategy of reverse genetics can be deployed, in which novel DNA-binding factors are isolated on the basis of their potential to bind regulatory elements of genes of interest (9, 17). The functional importance of these newly isolated factors in murine development is subsequently tested by generating mice carrying a targeted disruption of the gene coding for the factor of interest via homologous recombination in embryonic stem (ES) cells.
This report provides an overview of the data, generated in recent gene targeting experiments in mice which were designed to disrupt transcription factor genes. The majority of the transcription factors were first identified on the basis of their potential to bind cis-acting regulatory elements of genes that encode lymphocyte-specific transcription factors. Other transcription factors were cloned in retroviral studies or studies on translocations in leukemias or lymphomas. The functional relevance of these oncogenes for the transcription of lymphoid structural genes was recognized more recently.

More often than not, transcription factor gene disruptions perturb the development of multiple lineages. Often, this results in intrauterine death of the developing animal between embryonic day 9 and 16 due to severe anemia. Impaired development of tissues outside the hematopoietic system can also be the reason for embryonic lethality.

Nevertheless, a number of knock-out studies have directly demonstrated that some transcription factors are key regulators of lymphoid development. These factors are apparently uniquely required at different time points during the lymphoid development pathway. Mice carrying a mutation in a gene coding for such a factor are mostly viable and virtually normal except for having an impaired immune system. These mutant animals provide the researcher with bone marrow and lymphoid tissues with which experiments can be performed.

If the resulting mutant mice are not viable, several technologies are available to the researcher. 1) Mutant fetal liver stem cells derived from knock-out embryos can be cultured in vitro in B-lineage assays or in Fetal Thymic Organ Culture (FTOC) as a T-lineage assay. Alternatively, fetal liver cells can be grafted to lethally irradiated animals in reconstitution experiments. 2) Double knock-out (−/−) ES cells can be generated by performing two consecutive gene disruptions. These −/− ES cells can be tested directly for their lymphopoietic capacity in various in vitro assays. One further intriguing possibility when testing the lymphopoietic potential of −/− ES cells is to inject these cells into recombinase-activating gene 1 (Rag-1) or Rag-2 deficient blastocysts. Rag-1 and Rag-2−/− mice are characterized by the total absence of mature cells of the lymphoid system. Chimeric mice generated through the injection of −/− ES cells into Rag-1 or -2 blastocysts will therefore only have lymphocytes derived from the contribution of the −/− ES cells to the embryo.

Members of the HMG Domain Family

Tcf-1 and Lef-1 are prominent members of the HMG domain family of transcription factors. These proteins are highly homologous and are thought to have arisen through a recent gene duplication event. TCF-1 was originally defined and cloned on the basis of the affinity of its product for a defined motif in the CD3e enhancer (38). Lef-1 has been demonstrated to bind and regulate the TCRα enhancer (36, 41). During murine embryogenesis, the expression patterns for Tcf-1 and Lef-1 are complex and overlap to a large extent. Both genes are expressed in a variety of non-hematopoietic tissues, including kidney, lungs, and ribs (25). In adult animals, Tcf-1 expression is confined to T lymphocytes, whereas Lef-1 is expressed in T cells and in early B-lineage cells. In a homologous recombination experiment in which the Tcf-1 gene was disrupted, it was demonstrated that in the absence of the Tcf-1 gene product a severe block in early T-cell differentiation occurred. The block was observed at the immature single positive (ISP) stage. The ISP cell represents an intermediatory stage of T-cell differentiation in which the maturing T-lymphocyte expresses the pre-TCR containing the rearranged TCRβ chain and the pre-Tα chain, but has not yet rearranged the TCRα gene. In normal mice, the ISP cells are actively proliferating, but in the Tcf-1−/− mouse they are small and non-cycling. The T-cell phenotype of these mice is highly specific; the other hematopoietic lineages, including the B- and NK-cell lineages, are unaffected (40). Otherwise, the Tcf-1−/− animals are completely healthy.

Lef-1−/− animals, despite the clearly demonstrated role of Lef-1 in TCRα gene regulation, have a normal immune system. In contrast, these mice display an early neonatal phenotype: they lack teeth, mammary glands, sweat glands, hair and the trigeminal nucleus in the brain (39). No obvious defects were observed in
lymphoid populations at birth, possibly due to the fact that Tef-1 may take over the function of Lef-1 in these tissues.

The Sox-4 gene is closely related to the HMG box gene Sry, which codes for the mammalian sex-determining protein. The Sox-4 gene is expressed at multiple sites during embryonic development, including the central nervous system, the heart and lungs. Its adult pattern of expression is restricted to immature B and T lymphocytes. Sox-4/−/− embryos die in utero from circulatory failure at day E14, displaying a so-called hydrops foetalis. This is the direct consequence of the abnormal development of the heart. The aorta and pulmonary artery fail to separate properly, and the semilunar valves remain severely hypoplastic. To study lymphopoiesis in a mutant background, lethally irradiated mice were transplanted with Sox-4/−/− fetal liver cells. B-cell development was severely impaired at the pro-B cell stage in the recipient animals. In line with these in vivo observations, the frequency and proliferative capacity of IL-7-responsive B-cell progenitors were found to be severely diminished when fetal liver cells were subjected to an in vitro pro B-cell assay. No differential blocks were seen in any of the other hematopoietic lineages, including the T lineage (28).

Ets-1

The Ets-1 protein was first discovered as a predominantly lymphoid-expressed protein and it is thought to regulate lymphocyte-specific gene expression. Ets-1 is the cellular homologue of the viral v-ets gene, the founding member of the Ets transcription factor family (18, 20). The Ets-1 protein is phosphorylated upon cell activation. This phosphorylation presumably mediates its inactivation. Two groups recently reported the outcome of Ets-1 gene disruption experiments. In both cases, the phenotype was tested in the RAG-2−/− complementation system, because disruption of the Ets-1 gene probably results in embryonic lethality. In the chimeric mice, T cells were reduced in numbers, and displayed a high susceptibility to apoptosis (6, 23). Concurrently, double-positive (DP) and mature single-positive (SP) thymocytes were decreased in numbers. It is at present unclear whether this is the result of either a block in development or an increase in programmed cell death of DP thymocytes. B-cell numbers in the spleen appeared unaffected. A relative increase in mature B cells was observed. In particular, high numbers of Ets-1−/− IgM-secreting plasma cells were apparent. Class-switching was not affected. Expression of the putative target genes encoding the variable chains of the TCR and immunoglobulin (Ig) heavy (H)-chain were, surprisingly, not changed in the mutant lymphocytes. Thus, the target genes for Ets-1 that are directly responsible for the observed lymphoid phenotype remain to be elucidated.

Ikaros

Ikaros was originally identified on the basis of its ability to bind the CD36 enhancer (11). Its expression is predominantly confined to fetal thymus and the early fetal liver and, at later stages, appears to be restricted to T- and B-lineage lymphocytes. Ikaros encodes a zinc-finger protein with limited homology to the Drosophila gene Hunchback. A targeted disruption of the Ikaros gene resulted in a mouse with complete block of lymphoid development. No T-, B-
or natural killer (NK) cells are found in the severely immunocompromised mutant mice. The other hematopoietic lineages are relatively unaffected. It is hypothesized that the Ikaros protein fulfills a checkpoint function at the entrance to the three major lymphoid pathways (12). Ikaros also plays a role later in T-cell development: mice carrying a single mutant allele show generalized lymphoproliferative abnormalities, which, with age, erupt into clonal T-cell malignancies. These neoplasms are unique in that they apparently loose the wild-type Ikaros allele (42). Thus, Ikaros is also actively involved in the control of T-cell proliferation in an as yet, undetermined fashion.

E2A

Virtually all B-lymphocyte-specific genes are characterized by the presence of so-called E boxes in their regulatory sequences (15). The E box is bound by a variety of proteins of the basic Helix Loop Helix (bHLH) protein family of transcription factors. The E2A gene encodes such bHLH proteins, of which E47 and E12 are generated from alternatively spliced mRNAs. E12 and E47 differ in their homo- and heterodimerization potential. Although E47 polypeptides can be detected in all cell types, DNA-binding homodimers of E47 are found specifically in B cells (4, 31). Original studies on the potential role of E47 in controlling the B-cell phenotype indicated that its transfection into an early T-cell line could induce rearrangement and expression of endogenous immunoglobulin genes at a low frequency (29). The E2A gene was directly implemented in the control of B-cell differentiation in two recent gene disruption experiments. B cells were completely absent from mice carrying two disrupted E2A alleles, while T cells and all other hematopoietic lineages were present in normal numbers and phenotype (3, 43). This was very surprising given the ubiquitous expression of the E2A-encoded proteins. The B-cell differential arrest in the E2A-deficient mice occurs prior to the onset of immunoglobulin gene rearrangement. A complete disruption of the E2A gene completely prevents the accumulation of detectable B220 progenitor cells (43). In the second gene disruption experiment the alternatively spliced exon encoding the E12 bHLH domain was targeted. This also somehow impairs the expression of the E47 protein, but resulted in the appearance of some B220+ B-cell progenitor cells. These cells, however, were incapable of undergoing Ig gene rearrangements (3). The null mutation in the E2A gene resulted in a 50% reduction in pro-B cells in the bone marrow in heterozygous mice. The authors concluded that a dosage effect on levels of expressed E2A-encoded proteins underlay this curious reduction in B-cell numbers.

Id gene family

The Id genes encode helix-loop-helix proteins that, through dimerization with bHLH proteins, keep the latter from binding DNA and thus from exerting their normal function in gene regulation (5). Of these inhibitory Id genes, the Id1 and Id2 genes are expressed at high levels in pro-B cells. Id gene expression decreases with development along the B-lineage pathway. In an experiment in which transgenic mice were forced to express the Id1 gene throughout development along the B lineage, a crucial function of Id proteins was revealed (35). The transgenic mice contained dramatically decreased numbers of B-lineage cells, presumably due to sequestering of DNA-binding E12/E47 proteins, and consequently the down-regulation of E12/47-controlled B-target genes. The phenotype would thus be comparable to that of the E2A gene knock-out.

BSAP

The Pax5 gene encodes the BSAP protein. This protein contains a so-called “paired box” DNA-binding domain (1, 24). The paired box, as occurring in various members of the Pax family, recognizes a relatively long DNA motif: it can be divided into two separate protein domains, and thus constitutes a bipartite DNA-binding domain. BSAP was first found to control the expression of the CD19 gene in the B-cell lineage. BSAP carries various other names, including Sa-BP, HFB-HB and NF Sm-B1. BSAP is supposedly involved in class-switch recombination of the immunoglobulin heavy chain locus. Like the HMG box genes discussed above, BSAP is not only expressed in the B lineage, but also outside the hematopoietic system. i.e., in brain and testis. Targeted disruption of the Pax5 gene completely blocked B-cell differentiation, but left all other hematopoietic cell
lineages intact (37). In the bone marrow of Pax5−/− mice, pro-B cells exist that have undergone D-J rearrangement. However, the B cells fail to complete V-D-J gene rearrangement. BSAP has also been suggested to repress the activity of the Ig 3′ enhancer. It cannot be deduced from these mice if this is actually the case, since this aspect of BSAP function could not be analyzed in the knock-out mice owing to the absence of mature B-lineage lymphocytes.

CIITA

From patients with human bare lymphocyte syndrome (BLS), a hereditary severe combined immunodeficiency disease, two genes that specifically regulate MHC class II gene expression have recently been cloned by Mach and colleagues. One of these is the major histocompatibility complex (MHC) class II trans-activator (CIITA), which was cloned in a complementation assay using the MHC class II-negative cells from human patients with BLS (32). CIITA mediates both constitutive as well as interferon-γ (IFN-γ)-inducible expression of MHC class II genes. It also activates expression of the invariant (ii) chain, an MHC class II-associated protein involved in antigen presentation (7, 27, 32). Moreover, CIITA, upon transfection into myeloma cells and T cells, upregulates MHC class II expression on the cell surface.

The CIITA protein appears not to be able to bind to DNA. Rather, it is believed to regulate transcription as a coactivator upon interaction with specific (but as yet unidentified) DNA-associated proteins. The clinical syndrome is recapitulated in CIITA-deficient mice. These mice lack MHC class II on the surface of B cells and dendritic cells (8). Also, MHC class II gene expression cannot be activated on macrophages of these mutant mice by IFN-γ. Some MHC class II expression can be detected in thymus. Nevertheless, the CIITA−/− mice do not possess CD4+ T-helper cells, which resemble the phenotype of mice lacking a functional MHC I-Aβ gene as well as that of BLS patients.

RFX5

The second gene which was identified in BLS patients belongs to the RFX protein family, whose members represent the X box-binding proteins. The X box is an MHC class II-specific regulatory nucleotide sequence. Like CIITA, RFX5 was recently cloned by genetic complementation of an MHC class II− cell line derived from a patient belonging to a distinct BLS complementation group (33). Transfection of the RFX5 gene into the mutant cell line rescues MHC class II gene expression. As for CIITA, RFX-5 specifically regulates MHC class II gene expression. No other obvious abnormalities occur in BLS patients of this complementation group. No knock-out mice have been generated to date, although a prediction of the phenotype can be derived from that of these BLS patients.

EBF

EBF stands for Early B-cell Factor. It was initially defined as a pre-B and B-cell specific DNA-binding protein, regulating expression of the mb1 gene through an element in the promoter. The mb1 gene encodes the Igα protein, one of the constituent chains of the B-cell receptor. After cloning of EBF, it was found that this transcription factor did not fall into a known family of DNA-binding proteins. Nevertheless, EBF contains an HLH-like dimerization domain (13). In a gene disruption experiment, the Ebf gene was found to be crucial for early B-cell differentiation. As for several of the other factors discussed here, the T-lymphocyte and myeloid cell lineages were normally present (19). The phenotype of these mice resembled that of the weaker E2A knock-out allele. B-cell differentiation is blocked before the onset of D-J rearrangement of the Ig-H-chain locus. B-lineage cells were still present: the occurrence of B220+ cells with their Ig locus in germline configuration suggested that EBF is dispensable for commitment to the B-cell lineage, but that it plays a crucial role later during progression along the B-cell lineage.

Oct-2

Oct-1 and Oct-2 are two closely related POU-domain transcription factors. Oct-1 is ubiquitously expressed, whereas Oct-2 is expressed in a B-cell specific fashion. Oct-1 is believed to acquire B-cell specificity through its interaction with the B-cell-adaptor OBF-1 (24, 34). Knock-out of the gene encoding Oct-2 results in early neonatal death for undisclosed reasons. Terminal differentiation of B cells into plasma cells is strongly impaired. In particular, entry into the cell cycle, as induced by lipopolysaccharide
(LPS) stimulation, is deficient in Oct-2−/− B cells (10). Surprisingly, the expression of a large number of proposed B-cell-specific target genes (such as the immunoglobulin genes) is normal despite the absence of functional Oct-2 protein (10). Apparently, redundancy with other factors such as the Oct-1/OBF-1 complex masks the absence of Oct-2. By the application of a subtraction strategy utilizing B-cell lines derived from the Oct-2−/− mice transfected with Oct-2, CD36 was found to be a target gene critically dependent on Oct-2 gene control (16).

Perspectives

The study of the transcriptional regulation of structural lymphoid genes has in recent years been greatly facilitated by the cloning and characterization of transcription factor genes. Gene knock-out technology now provides the means to test the role of such transcription factors in normal lymphoid development. Previous biochemical studies, although useful, did not elucidate the functional role of individual factors in the genetic cascades determining lymphocyte fate and differentiation. The gene targeting experiments performed during the last few years have demonstrated that the disruption of single transcription factor gene often has dramatic effects on development and cellular differentiation. In several cases, as discussed in this review, it was shown that the absence of a single transcription factor can lead to complete loss of cellular development beyond the checkpoint controlled by that factor. In other cases, no obvious phenotypical changes were observed when a transcription factor gene was knocked out. These observations can often be explained by the redundancy effects of other, possibly closely related, proteins.

A major field of interest remains: What are the actual target genes that are controlled by these pivotal transcription factors? Technology to identify such target genes relies mostly on cumbersome subtraction techniques. Improvements are called for in order to unravel the molecular cascades.

Nevertheless, with the data already available and with forthcoming information, it will be possible to build a hierarchical genetic cascade for lymphoid development. Ultimately, the molecular control of every developmental checkpoint will be unraveled. The intricate interactions between the extracellular environment, surface receptors, signal transducers and transcription factors will become apparent. It is unlikely that the lymphoid genetic program in a lymphoid progenitor unfolds in an autonomous fashion. It is more plausible that every developmental step results from the integration of extracellular signals with genetic programs, such that changes in developing cells occur in harmony with their direct environment. Fascinating observations can be expected in this field in the near future.

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