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DIFFERENTIAL EXPRESSION OF GENES IN EMBRYOS LACKING SPECIFIC RETINOIC ACID RECEPTORS

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Retinoids, natural and synthetic derivatives of vitamin A, play a wide variety of biological roles in processes such as vision, reproduction and embryogenesis. The importance of retinoids in embryonic development was initially recognized in studies where Vitamin A-deficient dams produced offspring exhibiting a collection of developmental defects. To date, retinoids are known to be important in several embryogenic processes such as limb formation and axial skeletal patterning.

The effects of retinoids are mediated through retinoic acid, and retinoid-X, receptors (RARs and RXRs, respectively), which are members of the nuclear receptor superfamily. Both types of retinoid receptors are activated by 9-*cis*-RA, while only RARs are activated by all-*trans*-RA. RARs and RXRs are each encoded by three separate genes: alpha, beta and gamma. Additionally, there are N-terminal isoforms of the RAR genes. Upon ligand activation, RARs and RXRs form heterodimers and bind to specific retinoic acid response elements (RAREs) located in promoter and/or enhancer regions of target genes. The result is a modification of the rate of transcriptional initiation, presumably by interaction of the receptors with transcriptional machinery. By this mechanism, RARs and RXRs can cause a signaling cascade with the prospect of affecting numerous downstream genes, thereby controlling myriad biological processes.

When wild-type embryos are treated with retinoic acid at 8.5 d.p.c., a spina bifida phenotype is observed when they reach full term. Mice whose RAR(gamma) genes have been knocked out by homologous recombination do not exhibit this phenotype under identical experimental conditions. This strongly suggests that RAR(gamma) transduces the retinoic acid signal, which affects transcription of genes involved in normal pattering of the axial skeleton. However, the identities of these target genes remain to be discovered.

Differential display, a technique that utilizes reverse transcription and random amplification by PCR, identifies a "fingerprint" of genes expressed in a given cell or tissue type. The present study utilizes this

technique on whole embryos at 8.5 d.p.c. to find target genes that are transcriptionally regulated in wild-type, RAR(alpha)$_1$ null mutants and RAR(gamma)/(alpha)$_1$ double mutant mice in response to retinoic acid treatment. Genes that are truly differentially expressed in these different backgrounds will be cloned, sequenced and analyzed for homologies to known genes. Since RAR(alpha)$_1$ is expressed throughout most of the embryo, the use of double mutants should make the identification of RAR(gamma) target genes more clear by eliminating any effects of functional redundancy between the two receptors. This method has the potential of identifying known or novel genes that are direct or downstream targets of retinoic acid receptors. Time course analyses and gene dosage effects can also be readily studied. The findings of this project should lend greater understanding to retinoid signalling and the role of retinoids in developmental processes.

BIOGRAPHY

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INTERACTION BETWEEN TCR AND PURIFIED SINGLE MHC/PEPTIDE COMPLEX IS NOT SUFFICIENT TO INDUCE APOPTOSIS OF IMMATURE THYMOCYTES

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During intrathymic T-cell development, a high affinity/avidity interaction between (alpha)(beta) TCR on $CD4^+CD8^+$ thymocytes and MHC/self-peptide complexes on stromal cells leads to thymocyte deletion through apoptosis. The present investigators, and others, have previously shown that MHC/peptide complexes did not need to be expressed by a specific stromal cell type in order to induce thymocyte deletion. Therefore, it is conceivable that apoptosis can be induced through a TCR-mediated signal without requiring additional signals. The fact that CD3 cross-linking with immobilized monoclonal antibodies (MoAbs) triggers thymocyte apoptosis in the absence of stromal cells supports this view. However, reports have shown that the (alpha)(beta) TCR might not be functionally coupled to the CD3 complex on all $CD4^+CD8^+$ cells. In fact, it has been demonstrated the (alpha)(beta) TCR cross-linking via anti-TCR (beta)-chain MoAbs induces minimal DNA degeneration in thymocytes from (alpha)(beta) TCR non-transgenic mice. Nonetheless, concomitant stimulation of thymocytes by anti-TCRß-chain and anti-CD28 MoAbs increases the levels of DNA degradation in those thymocytes. Yet, MoAbs cannot mimic stimulation given by physiological ligands. Hence, in vivo stimulation through the (alpha)(beta) TCR is given by an MHC/peptide complex, which also cross-links co-receptors, such as CD4 or CD8, that may increase the avidity of the interaction and alter the intracellular signaling cascade. Also, the affinities of MoAbs to the (alpha)(beta) TCR are 10^4-10^5-fold higher than those of MHC/peptide complexes. The goal of the present study was thus to verify if purified

MHC/peptide complexes could induce apoptosis of thymocytes. Immobilized I-E^k molecules, loaded with a peptide of moth cytochrome C that could be recognized by thymocytes expressing a transgenic (alpha)(beta) TCR (AD10), were used. In this model system, it is shown that engagement of the (alpha)(beta) TCR of transgenic thymocytes by I-E^k/peptide complexes cannot induce apoptosis at concentrations able to stimulate T cell hybridoma or mature $CD4^+CD8^-$ thymocytes expressing the same (alpha)(beta) TCR. More importantly, it is demonstrated that simultaneous stimulation through CD28 and (alpha)(beta) TCR by anti-CD28 MoAbs and I-E^k/peptide complexes respectively, induces phenotypic changes associated with apoptotic death of thymocytes: namely, i) loss of membrane asymmetry, detected by MC540 staining; ii) up-regulation of CD69 expression, reminiscent of (alpha)(beta) TCR stimulation; iii) down-regulation of CD4 and CD8 expression; iv) increased DNA degradation revealed by TUNEL assay. Briefly, the results indicate that apoptosis can be induced following the stimulation of the (alpha)(beta) TCR by its natural ligands, thus at physiological affinities, when accompanied by CD28 engagement. Investigations are underway to determine whether this effect can be reproduced by engaging CD28 with B7 molecules, rather than MoAbs. The present investigators are also using this model-system to identify other co-stimulatory molecules that could synergize with (alpha)(beta) TCR-mediated stimulation to induce $CD4^+CD8^+$ thymocyte apoptosis.

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