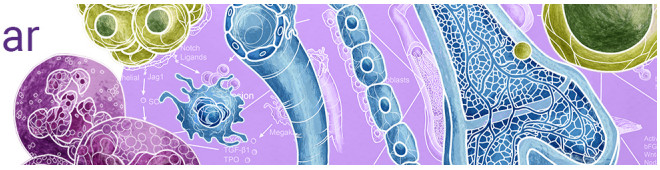

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THE JOURNAL OF IMMUNOLOGY

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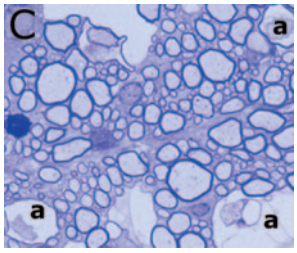
Novel CNS Autoimmunity Model



Studies of CNS autoimmunity indicate that immune cells other than T cells might be involved. Although overexpression of chemokine CCL2 within the CNS results in monocyte/macrophage accumulation, any link between those cells and neurological disease has not been established. Furtado et al. (p. 6871) found that all double-transgenic mice constitutively expressing CCL2 under control of the myelin basic protein promoter and conditionally expressing myeloid growth factor FMS-like tyrosine kinase 3 ligand (FLT3L) in response to doxycycline (DOX) treatment developed paralytic encephalomyelitis within 9 days of DOX treatment. Wild-type and single-transgenic controls or double-transgenic mice not given DOX remained healthy. Diseased CNS tissues exhibited massive infiltration of $CD11c^+$ mononuclear cells, and the number of circulating cells expressing dendritic cell (DC) and monocyte markers increased with DOX treatment. Elimination of $CD4^+$ and $CD8^+$ T cells by specific mAbs did not affect disease development. CNS disease was reduced by ~70% in DOX-treated mice injected with liposomes containing a compound that induced apoptosis of circulating monocytes and DCs. Suspension of DOX treatment after 10 days resulted in a chronic demyelinating neuroinflammatory disease with $CD8^+$ T cell infiltration. This novel model of acute CNS disease involves CCL2-induced infiltration of monocytes and DCs into the CNS of transgenic mice conditionally expressing FLT3L; chronic demyelinating disease with T cell involvement occurs after cessation of FLT3L expression.

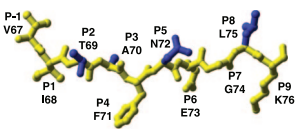
Regulating *CD1A* Transcription

Although expression of CD1a, a molecule that presents lipid Ag to a restricted set of T cells, is limited to a few cell types, factors regulating its expression are unknown. *CD1A* mRNA transcription was found by Colmone et al. (p. 7024) to correlate directly with protein expression levels on human T cells. Analyses of reporter constructs defined a 1000-bp fragment as sufficient and required for *CD1A* promoter activity. Single base pair mutations in four transcription factor binding sites identified by computer search programs resulted in decreased promoter activity. Proteins binding *CD1A* were detected by EMSA on nuclear extracts of human T leukemia cells and identified as CREB-1 and activating transcription factor-2 (ATF-2) using specific Abs in supershift experiments and chromatin immunoprecipitation assays. *CD1A* promoter activity and mRNA levels decreased in cells treated with a cAMP activator that phosphorylated and activated ATF/CREB family members; increased promoter activity occurred in cells cotransfected with a vector expressing a dominant-negative CREB plus the *CD1A* reporter construct. Vector-expressed short hairpin RNA specific for *ATF-2* decreased *ATF-2* mRNA expression in T cell lines, whereas short hairpin RNA specific for *CREB-1* resulted in decreased expression of *CREB-1*, *ATF-2*, and *CD1A* mRNA. Chromatin immunoprecipitation assays using anti-CREB-1 or anti-ATF-2 Abs with primary human PBMC extracts showed more binding of ATF-2 in $CD1a^-$ monocytes compared with $CD1a^+$ monocyte-derived dendritic cells. Thus, ATF-2 and CREB-1 regulate *CD1A* transcription by direct binding to its promoter in human cells in vivo and in vitro.

Limiting TB in T Cell-Deficient Hosts

Although production of IFN-γ by T cells controls *Mycobacterium tuberculosis* infection, any contribution of IFN-γ produced by NK cells is unknown. Feng et al. (p. 7086) detected high levels of intracellular IFN-γ in naive NK cells from $RAG^{-/-}$ mice stimulated in vitro with live *M. tuberculosis* and in NK cells from lungs of mice infected 4 wk earlier. Whereas $RAG^{-/-}$ mice were able to survive infection longer than 40 days postinfection, $IFN\text{-}\gamma^{-/-}$ mice or $RAG^{-/-}$ mice lacking NK cells or IL-12p40, or treated with anti-IFN-γ mAb, died by 30 days. Survival rates correlated inversely with number of bacteria, number and size of lesions, and number of infiltrating granulocytes in their lungs. Exogenous IL-12 restored the IFN-γ response to bacteria-stimulated splenocytes from $RAG^{-/-}$ animals lacking IL-12p40 or IL-12p35 but not to those lacking NK cells. Lung pathology was more severe in $RAG^{-/-}$ animals lacking IL-12p40 vs IL-12p35. Only infected $RAG^{-/-}$ mice up-regulated NO synthetase type 2 expression in pulmonary macrophages, whereas modulators of its expression were down-regulated compared with the other mutant strains or with anti-IFN-γ mAb-treated $RAG^{-/-}$ controls. Neutrophil chemotactic chemokine production was elevated in infected $RAG^{-/-}$ mice lacking IL-12p40 or treated with anti-IFN-γ mAb, whereas bacterial titers and lung pathology were increased in infected anti-IFN-γ mAb-treated $RAG^{-/-}$ mice depleted of neutrophils. The authors propose that IFN-γ produced by NK cells cooperates with IL-12 to protect mice lacking T cells against *M. tuberculosis* infection via activation of macrophage microbicidal activity and neutrophil infiltration.

TCR Specificity and Degeneracy



Although TCR recognition of a peptide/MHC is specific, there also is degeneracy in that altered peptide ligands containing a single amino acid substitution at a TCR contact residue can be recognized. This duality was explored by Donermeyer et al. (p. 6911) in a mouse model Ag system specific for a hemoglobin peptide/MHC class II complex. Two high-affinity TCRs,

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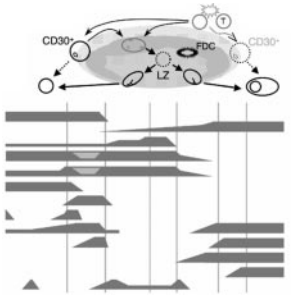
evolved in vitro from a wild-type TCR by yeast surface display, accumulated four plus an additional three mutations in their CDR loops, respectively. A series of hemoglobin peptide variants was constructed by singly substituting 1 of 20 aa at each of the four TCR contact residues, P2, P3, P5, and P8. TCR-transfected cells recognized more P2, P3, and P5 mutants with TCR evolution from wild type, to intermediate, to high affinity. A high degree of specificity was retained for recognition of peptides altered at P8. Degenerate peptide binding with the high-affinity TCR was estimated to be 800-fold stronger than low-affinity interaction of wild-type peptide with wild-type TCR. Even though a related nonstimulatory moth cytochrome c peptide had permissible hemoglobin amino acids at P2, P3, plus P8, it was nonstimulatory for any of the TCRs. Chimeric hemoglobin/moth peptides in which moth MHC contact residues P4 plus P6 or P7 alone were substituted into the hemoglobin peptide were stimulatory, but the three substitutions together were not. The experiments demonstrate that high-affinity TCRs maintain peptide specificity but become degenerate for additive changes in peptide TCR contact residues or in MHC contact residues that may impact peptide conformation.

Factoring B Cell Maturation

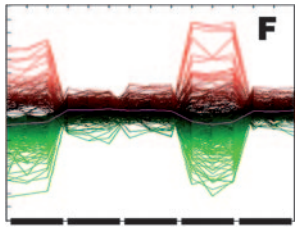


Activation and repression of a variety of transcription factors occurs during transit of activated B cells through the germinal center (GC) and in subtypes of diffuse large B cell lymphomas. In their detailed analysis of transcription factor expression using double immunofluorescence on single cells, Cattoretti et al. (p. 6930) compared late B cell lymphopoeisis in mouse spleens and human tonsils. B cell chronic lymphocytic leukemia/lymphoma 6 (BCL6) and IFN response factor 4 (IRF4) had mutually exclusive expression at the mRNA and protein levels: the former, along with PU.1, IRF8, and B cell-specific activator protein 5 (Pax5), was expressed highly in GCs, whereas the latter was expressed in mantle and marginal zone B cells. $CD30^+$ extrafollicular blasts were positive for IRF4 and Pax5 with inverse intensities. IRF4 expression in activated activation-induced cytidine deaminase (AID^+) extrafollicular blasts was lost when cells entered the GC but reappeared with B lymphocyte-induced maturation protein 1 expression after commitment of interfollicular and intrafollicular cells to the plasma cell lineage. Each B cell subset had a characteristic transcription factor protein expression level and pattern. BCL6 and IRF4 coexpression patterns were diverse among 40 diffuse large B cell lymphomas, and, in tumors of putative GC origin, a specific IRF4 conformational epitope masked in normal B cells was detected. This use of single-cell analyses of human and mouse B cells delineates expression patterns of key transcription factors during the GC reaction and in malignant B cells.

Two Pathways to DNA Vaccination

Developing potent $CD8^+$ T cell memory responses, a challenge in DNA vaccination strategies, is dependent on $CD4^+$ T cell help at priming. To optimize vaccine design, Radcliffe et al. (p. 6626) compared the Ag presentation pathways of two fusion DNA vaccines. They injected naive mice with *pDUO* (a DNA plasmid carrying an endoplasmic reticulum-targeted OVA peptide fused to a core peptide sequence from the N-terminal domain of tetanus toxin fragment C inserted into the mouse invariant chain to replace the CLIP sequence). The animals had several-fold higher numbers of IFN-γ-producing T cells compared with mice vaccinated with the OVA peptide fused to the toxin fragment alone (*pDOM-SL8*). $CD4^+$ T cell depletion reduced the *pDUO* and *pDOM-SL8* responses; $CD8^+$ T cell expansion was greater and lasted longer with *pDUO* vaccination. Transfer experiments with naive CFSE-labeled OVA peptide-specific TCR transgenic cells showed that OVA peptide from injected donor cells transfected with *pDUO* was presented directly by cells that matched the MHC of the mice; OVA peptide from *pDOM-SL8* transgenic cells was cross-presented by MHC mismatched cells as well, even in the absence of TAP2. Mice primed with *pDUO* and challenged 50 days later with *pDOM-SL8*, or vice versa, had higher memory and cytolytic $CD8^+$ T cell responses than mice boosted with the priming vaccine. The authors demonstrate that alternating direct and cross-presentation of Ags delivered by DNA vaccines results in heightened effector and memory $CD8^+$ T cell responses.

Human Monocyte/Macrophage Transcriptome



Although gene expression profiles for differentiation of mouse monocytes to macrophages are available, preliminary information about human mononuclear cell differentiation indicates important species differences. Detailed oligonucleotide microarray analyses of primary human cells by Martinez et al. (p. 7303) indicated that M1 polarization induced by LPS plus IFN-γ treatment of M-CSF-differentiated macrophages was accompanied by significant early modification of the monocyte maturation global transcriptome; macrophages polarized to M2 by IL-4 treatment had only subtle adjustments to the transcriptome. Gene ontology analyses of gene clusters identified in monocyte-to-macrophage differentiation showed changes in proteins involved in cell cycle, lipid metabolism, and immune response. M1 polarization was accompanied by massive changes in transcripts associated with classical macrophage activation, including DNA transcription, protein metabolism, and proinflammatory cytokines; M2 polarization was accompanied by less dramatic changes in cytokines, chemokines, and scavenger receptors. G protein-coupled receptor repertoires varied with the stage of monocyte differentiation and macrophage polarization. This global profiling approach identifies stage-specific genes modulated during human macrophage differentiation and polarization and points to differences with the mouse profile.

Summaries written by Dorothy L. Buchhagen, Ph.D.