production (oxidation of cytochrome c) were measured. At optimum antigen:antibody ratio, adalimumab and infliximab formed huge complexes >30 nm in diameter, suggesting that these bivalent antibodies crosslink TNF trimers. Certolizumab pegol complexes were <20 nm in diameter, consistent with its univalent structure preventing crosslinking. Certolizumab pegol bound 2.9 monomers at saturation, whereas infliximab and adalimumab both bound around 2.5 monomers. This suggests that steric or allosteric restrictions prevent the bivalent antibodies binding to all monomers in a trimer. The large immune complexes formed by adalimumab and infliximab caused degranulation and superoxide production by neutrophils. The smaller certolizumab pegol complexes had only marginal effects. The bivalent structures of infliximab and adalimumab form enormous complexes with TNF trimers, which have proinflammatory effects on neutrophils *in vitro*. Certolizumab pegol does not form large complexes with TNF trimers due to its univalent structure preventing crosslinking, giving it a unique mode of action in this regard.

doi:10.1016/j.clim.2007.03.124

Su.86 Certolizumab Pegol and Adalimumab Accumulation in the Inflammed Paws of Mice with Collagen-induced Arthritis Compared to Noninflammed Tissue *In Vivo* Biofluorescence Imaging of Alexa 680-labeled Antibodies

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Variability in disposition of monoclonal antibodies results in different exposure time courses in inflammed and noninflammed tissues. We investigated the disposition of certolizumab pegol and adalimumab in normal and inflammed tissue *in vivo* in mice, using a novel noninvasive biofluorescence technique. Certolizumab pegol or adalimumab labeled with the low-molecular weight dye Alexa 680 was administered intravenously (2 mg/kg) in naïve DBA/1 mice and in DBA/1 mice with ongoing collagen-induced arthritis. The accumulation of certolizumab pegol and adalimumab was measured in the hind paws at multiple points up to 26 hours using a Xenogen IVIS200 biofluorescence imager. Tail blood samples were taken for determination of serum levels of the reagents by ELISA. Both reagents penetrated inflammed tissue more than noninflammed tissue. The penetration of certolizumab pegol into inflammed arthritic paws was greater and more prolonged than for adalimumab (inflammed:noninflammed tissue ratios were 3.9:1 for certolizumab pegol and 1.9:1 for adalimumab; elimination half-lives were 27.8 and 5.5 hours, respectively). The plasma time courses reflected known differences in exposure, with certolizumab pegol maintaining higher plasma concentrations than adalimumab. In this model, certolizumab pegol disposition was more responsive to inflammation, with prolonged tissue infiltration occurring primarily in inflammed tissue. Certolizumab pegol, in contrast to adalimumab, appeared to readily enter inflammed tissue but not noninflammed tissue. This feature of certolizumab pegol may be conferred on the molecule by PEGylation. Increased drug exposure at the site of inflammation might be an important consideration for the treatment of inflammatory disorders such as rheumatoid arthritis and Crohn's disease.

doi:10.1016/j.clim.2007.03.126

Su.87 Comparison of Naturally Occurring Human Immunoglobulin Sequences with the Anti-TNF Agents Certolizumab Pegol, Adalimumab, and Infliximab

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V-region sequences of certolizumab pegol, adalimumab, and infliximab were compared with a database of normal human IgG V-region sequences. A database of rearranged human variable heavy [VH] and variable kappa light [VK] amino acid sequences was obtained by sequencing 243 unique VH chains and 312 unique VK chains from healthy donors. Each sequence was compared to the closest human "germline" sequence and also scored against every other sequence of the same chain type, using percentage identity to measure sequence similarity. Z-scores were calculated from a frequency distribution plot and compared between agents. A positive Z-score indicates that a sequence is more typical than average of a human sequence. The mean number of nongermline framework residues in rearranged human antibodies was 4.3% for VK and 8.8% for VH. Of the three agents, certolizumab pegol had a V-region most typical of the rearranged human sequences with 3.8% nongermline residues for VK and 8.5% for VH. Adalimumab had 1.3% and 1.2% and infliximab 31.3% and 22.2%, respectively. Positive Z-scores were obtained for the VK and VH sequences of certolizumab pegol (0.15 and 1.46) and adalimumab (0.67 and 1.34). However, infliximab V-region sequences gave negative Z-scores (−2.37 and −0.58) indicating they are less typical of human sequences. The certolizumab pegol V-region framework sequence has a similar mean number of nongermline residues as rearranged human IgG V-region

frameworks. The complete VH and VK sequences were also more human-like than average. The certolizumab pegol Fab' sequence can be considered typical of naturally occurring human IgG.

doi:10.1016/j.clim.2007.03.127

Su.89 Role of Enteric Lumenal TLR Ligand Sampling in the Formation of Novel Resident Mucosal Dendritic Cells

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Resident intestinal dendritic cells (DCs) play important immunoregulatory roles through sensing and antigen handling of enteric microbiota. However, intestinal DCs have not been well characterized. In this study, we found that CD11c+ cells isolated from the small intestine superficial and deep lamina propria were unusual (compared to lymph node and spleen DC's) due to their graduated expression of CD11b or B220, and the predominance of B220+CD11b+ double-positive (DP) DC's. Since lamina propria DC's engage in lumenal sampling and thus encounter TLR ligands, we wondered whether TLR signaling might induce the unusual DP phenotype. Using bone marrow-derived DC's differentiated with Flt-3L, we evaluated the effect of co-culture with various TLR ligands. Whereas Flt-3L alone induced single-positive CD11b+ or B220+ DCs, DP DCs predominated after TLR2, TLR4, and TLR9 ligands. The response was abrogated in myd88−/− mice, suggesting that DP DC's are induced through TLR-MyD88 pathway. Fecal extracts also induced DP DC formation; because this response was absent in TLR4−/− mice, it appeared that the predominant enteric bioactivity for DP DC induction was LPS. Among lamina propria DCs, we also observed a probable immature mDC subset (CD11c-B220-CD11blow). This subset was deficient in germ-free (GF) mice or cd8 knockout mice, suggesting an important TLRL role in differentiation stage of DCs *in vivo*, and a novel requirement for CD8+ T cells. These findings indicate that lumenal TLR4 ligands shape the differentiation of mucosal resident DCs, resulting in a novel phenotype associated with distinct immunoregulatory traits. Supported by NIH DK46763 and DK69434.

doi:10.1016/j.clim.2007.03.128

Su.90 Up-Regulation of the PI3-Kinase Pathway in Lamina Propria T Lymphocytes

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Understanding the regulation of immune responses in the normal intestinal mucosa is critical for defining its alterations in inflammatory bowel disease. Importantly, intestinal lamina propria T lymphocytes (LPT) when investigated *ex vivo* exhibit functional properties profoundly different from those of peripheral blood T lymphocytes (PBT). In particular, following CD2 stimulation they are able to produce markedly higher levels of cytokines than PBT. This study provides insight into signaling events associated with the high CD2 responsiveness of LPT. CD2 stimulation results in enhanced phosphorylation of the PI3-kinase dependent kinase Akt and its down-stream target GSK-3 in LPT when compared to PBT. That up-regulation of PI3-kinase pathway activation in LPT contributes to the high IL-2 expression in response to CD2 stimulation is demonstrated by the fact that inhibition of this pathway by the PI3-kinase specific inhibitor Ly294002 to levels induced in PBT clearly reduces IL-2 gene expression (and TNF-α, CD40L gene expression) in LPT. Immunohistochemical analysis reveals that Akt phosphorylation occurs in few lamina propria mononuclear cells in the normal mucosa *in vivo*; more intense phospho-Akt staining of larger numbers of lamina propria mononuclear cells is detectable in the inflammed intestine (ulcerative colitis) indicating that activation of the PI3-kinase pathway plays an important role in intestinal inflammation. Enhanced responsiveness of these pathways to costimulatory stimuli may allow for rapid and vigorous T cell responses and could therefore be fundamental to intestinal mucosal immune responses and homeostasis.

doi:10.1016/j.clim.2007.03.129

Su.91 Differential Levels of Intercellular Glutathion (GSH) Dictate the Shift From Adaptive to Innate T Cell Function in the Human Intestinal Mucosa

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Isolated human T cells from the healthy human mucosa are non-responsive to T cell receptor engagement yet mount