induced fluorescence. Parallel processing speeds up handling and improves reproducibility. Picomolar concentrations can be quantified at precision levels ~ 5-10% CV.
Miniaturization reduces volume requirements for sample and reagent. A protein is quantified using <1 µl of sample. Potentially >10 different proteins could be quantified simultaneously from a 20 µl sample, increasing information content and providing results within hours.

75.31

Role of macrophages in traumatic skeletal muscle injury

Mukesh Summan, Tracy Hulderman, Joanna M Matheson, Petia P Simeonova. Toxicology and Molecular Biology, DHHS/CDC/NIOSH, 1095 Willowdale Road, Morgantown, WV 26505

Traumatic skeletal muscle injuries result in profound histopathological changes and loss of muscle function. These injuries are associated with local infiltration of large numbers of mononuclear cells, degeneration of injured myofibres and phagocytic removal of cell debris. In the present study we evaluated the role of systemic macrophages in the injury/repair mechanisms in a traumatic skeletal muscle injury model using liposome encapsulated clodronate, a drug with well characterized monocyte/macrophage depleting qualities. C57BL/6 mice (n = 4 per group) were injected with clodronate liposomes 48 and 2 hours prior to the freeze injury of the left tibialis anterior (TA) muscle and every third day during the post-injury period. Control mice received phosphate buffered saline (PBS) liposomes. At 1, 3 or 9 days post-injury, the TA muscles were harvested for histology or gene expression evaluation by quantitative real time RT-PCR. Histopathological examination revealed less inflammatory cell infiltration in the injured muscles of clodronate treated mice at day 3 post-injury and delayed muscle tissue recovery with an impaired clearance of the necrotic myofibers at day 9 post-injury. Furthermore, macrophage depletion significantly attenuated injury-induced inflammatory cytokine and growth factor mRNA expression, for example tumor necrosis factor α, when compared to the PBS-liposome treated mice. These findings define the role of macrophages and the related cytokines as critical components of the complete recovery from skeletal muscle traumatic injury.

75.32

Reversal of Erosive Changes with Addition of Infliximab to Standard Therapy in Rheumatoid Arthritis

David H Snow. Carolina Arthritis Associates, PA, 1710 S. 17th Street, Wilmington, NC 28401

Infliximab (IFX) in combination with methotrexate (MTX) significantly improves signs and symptoms of rheumatoid arthritis (RA), improves physical function and inhibits progression of structural joint (JT) damage. We describe reversal of erosive (ERO) changes when IFX was added to standard therapy (TX) in a refractory RA patient (pt). A 67 year old white male with a 3yr history of RA had impressive synovitis of metacarpophalangeal (MCP), proximal interphalangeal (PIP) and subtalar JT, wrists, ankles, and knees. Radiographic (RG) evidence revealed extensive ERO changes of PIP, joint space narrowing (JSN) of MCP, and erosions of IP. Previous TX with prednisone (GC), NSAIDS, HCQ, MTX, Aurothioglucose (AU), and SSZ proved inadequate. Synovitis and elevated ESR persisted with increasing weekly doses of MTX. After 3 infusions of 3mg/kg IFX, synovitis of MCP and PIP resolved and fist function was restored to normal. After 2 years, pt continues to receive 5.6 mg/kg IFXq8 wk with no serious infusion reactions or serious adverse events. Au TX was discontinued, GC and MTX doses decreased. Pt shows no evidence of synovitis in hands, wrists, feet, or knees. Repeat RG evaluations show healing of ERO changes and JSN of PIP, right first IP, and restoration of ulnar styloid process. Pt reports marked improvement in quality of life and physical function. This case report suggests that IFX added to standard combination therapy may promote reversal and healing of those erosive changes.

75.33

Interleukin 6 indirectly induces migration of cultured epidermal cells from IL-6 deficient mice

Randle Michael Gallucci, Dusti K Sloan, Anne B Murray, Sijy J O'Dell. Pharmaceutical Sciences, University of Oklahoma, P.O. Box 26901, Oklahoma City, OK 73190

We have previously shown that IL-6 deficient transgenic mice (IL-6KO) display significantly delayed cutaneous wound healing compared to wild type control animals, requiring up to three fold longer to heal. While the necessity of inflammation during wound healing has been well established, the role of IL-6 in this process has not. To further describe the role of IL-6 in skin wound healing, an in vitro model was developed utilizing cultured dermal keratinocyte and epidermal fibroblast cells from neonatal IL-6KO mice. This system allows for the direct assessment of the effects of IL-6 on skin cells without the confounding presence of endogenous IL-6. Using a transwell migration assay, we have found that IL-6 appears to significantly induce cell motility in cultured IL-6KO keratinocytes (up to 5 fold) when cultured in the presence of dermal fibroblasts. In addition to these functional data, results from gene array analysis of IL-6 treated fibroblasts indicate that IL-6 does not appear to modulate any known soluble keratinocyte migratory factors. These data indicate that a likely mechanism by which IL-6 can modulate wound healing is by stimulating the migration of keratinocytes indirectly through the production of an as yet unidentified soluble fibroblast derived factor.

75.34

Automation of intracellular cytokine staining for flow cytometry

Laurel Nomura[1], Holden T. Maecker[1], Pierre Bierre[2]. [1]BD Biosciences, Immunocytometry Systems, 2350 Qume Dr, San Jose, CA 95131, [2]PB Consulting, pbierre@attbi.com

Flow cytometric analysis of intracellular cytokine responses offers a way to measure immune function within specific cell types (e.g. CD4, CD8 T-cells). After activation of whole blood samples, cells are prepared for cytokine flow cytometry (CFC) by lysing, centrifugation washing, permeabilizing, rewashing, staining with mAb, and washing again. Our goal was to adapt a commercially available, cell-washing test-tube robot, BD Biosciences' Lyse/Wash Assistant (LWA), to automate these sample preparation steps. The LWA with Intracellular Option handles all the above steps except mAb dispensing. The LWA first runs a *pre-staining protocol*, which performs cell lysis and permeabilization. Samples are then removed for manual mAb addition. Finally, the samples go back on the LWA for a *post-staining protocol*, which times the mAb incubation and performs a final wash, outputting analysis-ready cells. Equivalency with the manual CFC method was established through regression analysis on two key results variables: percent of CD69+ cytokine+ cells, and population mean signal-to-noise. Lab worker hands-on time was reduced by about 80%. The LWA is very simple to operate, and with a few modifications for intracellular staining, offers a low-cost method for standardizing sample preparation of cytokine flow cytometry assays. Research supported by BD Biosciences, Immunocytometry Systems.

75.35

Role of CCL3 in Protective Antiviral Immunity

John M Dye[1], Allan J. Zajac[2], Daniel G Quinn[1]. [1]Microbiology & Immunology, Loyola University, 2160 South First Avenue, Maywood, IL 60153, [2]Microbiology, Univ. of Alabama at Birmingham, Birmingham, AL

We have shown that mice deficient in the chemokine CCL3 or its receptors fail to develop protective immunity against secondary intracranial (i.c.) infection with lymphocytic choriomeningitis virus (LCMV). Immunostaining of brain sections revealed no difference between the accumulation or localization of CD8 cells in the brains of i.c. infected CCL3-deficient mice compared with B6 mice. This suggests that the failure of CCL3-deficient mice to control the infection is not due to a failure of the CD8 cells to localize to the infected CNS. By flow cytometry we found that all of the LCMV-specific CD8 cells and approximately half of the LCMV-specific CD4 cells express CCL3. We investigated whether CCL3 production by infiltrating immune cells

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International Society for NeuroImmunoModulation
International Society of Neuroimmunology
PsychoNeuroImmunology Research Society
Society for Leukoctye Biology
Society for Mucosal Immunology
Society for Natural Immunity

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Editor-in-Chief
Boyer Center for Molecular Medicine
Yale University School of Medicine
295 Congress Avenue, (P.O. Box 9812)
New Haven, CT 06519-1418, USA
Phone: 203-737-2334 Fax: 203-737-2267
email: vincent.marchesi@yale.edu

Editorial Office
Boyer Center for Molecular Medicine
Yale University School of Medicine
295 Congress Avenue (P.O. Box 9812)
New Haven, CT 06519-1418, USA
Phone: 203-737-2334 Fax: 203-737-2267
Email: faseb@yale.edu

Publications Office
The FASEB Journal
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Bethesda, MD 20814-3998, USA
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