by which p65 and GR interact to antagonize each other's function we employed p65 RHD, a dominant negative regulator of p65 transactivation, in transient cotransfection assays. Interestingly, p65 RHD efficiently blocks p65 transactivation of an NF-κB responsive reporter, yet cannot interfere with p65 mediated repression of GR transactivation and has no direct effect on GR transactivation (despite the fact that GR and p65 RHD co-IP). This indicates that the transactivation domain of p65 is required for repression of GR transactivation, but that NF-κB need not be transcriptionally active to repress GR. Cotransfection studies in which CBP is overexpressed indicate that both GR-mediated repression of p65 transactivation and p65 mediated repression of GR transactivation are dose-dependently enhanced by CBP, suggesting that the mechanism of p65-GR antagonism is not a simple competition for a limiting pool of CBP, but rather that CBP might serve as an integrator for p65 and GR which facilitates physical interaction between the DNA-bound transcription factors. Studies using the glucocorticoid antagonist RU 486 indicate that antagonist-bound GR, though transcriptionally inactive, retains the ability to interact with p65 and repress its transactivation. However, antagonist bound GR does not co-IP with CBP. Consequently, CBP cannot enhance the physical interaction of GR with, or the repressive effect of antagonist-bound GR on, NF-κB.

663 PROTECTIVE EFFECT OF RECOMBINANT INTERLEUKIN-6 ON SKIN INFLAMMATION CAUSED BY 7,12-DIMETHYLBENZ[A]ANTHRACENE IN INTERLEUKIN-6 NULL MICE.

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Interleukin-6 (IL-6) is a multifunctional cytokine, which regulates essential physiological functions such as acute phase reaction, immune response, hematopoiesis and bone metabolism. In order to examine the protective effect of IL-6 on skin damage caused by 7,12-dimethylbenz[*a*]anthracene (DMBA), we have conducted administration of IL-6 extracorporeally to the IL-6 null (IL-6-/-) mice. Female IL-6-/- mice and wild type B6J129Sv mice were topically applied a single dose of DMBA (500μg /mouse) on dorsal skin. Osmotic pumps with a delivery rate of 0. 5 μl/h were filled with recombinant human IL-6 (rhIL-6) (10 or 25μg/day) and then implanted subcutaneously on the ventral side of the mice (IL-6-/- + rhIL-6 mice). In control mice, PBS was filled instead of rhIL-6. Tissue samples were collected 5 days after DMBA administration, and subjected to histopathological examination. A severe skin damage was observed in IL-6-/- mice characterized by lymphocyte infiltration and keratinocyte hyperproliferation, whereas only epidermal hyperplasia was observed in the wild-type mice. Recombinant hIL-6 treatment to DMBA-treated IL-6 null mice suppressed the ocurrence of the above-mentioned skin damage. The present results suggest that IL-6 plays an important role in defense mechanism for cutaneous inflammation caused by DMBA.

664 ELEVATED OXIDATIVE STRESS IN SKIN OF B6C3FL MICE AFFECTS DERMAL EXPOSURE TO MACHINE WORKING FLUID.

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Metal Working Fluids (MWFs) are widely used for metal cutting, drilling, shaping, lubricating and milling in the industrial world. Potential for dermal and inhalation exposure to MWFs exists in a large number of workers via aerosols, splashing during the machining process, and other industrial operations. Both females and males are involved in these industrial operations. It has been reported earlier that occupational exposure to MWFs causes health problems, e.g. allergic and irritant contact dermatitis. Previously, we have shown that dermal exposure of B6C3F1 mice (female and male) to 5% MWFs for 3 months resulted in an increase in skin histamine and mast cell counts. Topical exposure to MWF also resulted in depletion of two major water soluble antioxidants, e.g. ascorbate and glutathione in the liver of both genders. The level of lipid peroxidation (MDA) in the liver of both sexes and the testes in males was concomitantly increased. The goal of this study was to evaluate interaction between oxidative stress in the skin and topical application of MWF. To create acute skin inflammation, an H_2O_2 producing enzyme, glucose oxidase conjugated to polyethylene glycol (GOD-PEG), was injected intradermally. Intradermal administration of GOD-PEG to B6C3F1 mice (female and male, 8-12 weeks old) resulted in inflammation and muscle fiber necrosis in the skin. The level of GSH in skin was reduced after GOD-PEG treatment of B6C3F1 mice. To determine whether oxidative stress influences the dermal response to MWF, mice were injected with GOD-PEG 24 h prior to topical exposure to unused MWF (200 μl 30%). This resulted in an increased inflammatory response in skin, reduced GSH and protein thiols, and an increased level of peroxidative products. Further study is necessary to delineate the role of oxidative stress in enhancing contact/irritant dermatitis triggered by occupational exposures to MWF.

665 GLUTATHIONE (GSH) QUENCHES PEROXYNITRITE - MEDIATED NITRATION OF PROSTAGLANDIN H SYNTHASE FORM-2 (PGHS-2).

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Increased expression of both inducible nitric oxide (NO) synthase and PGHS-2 has been reported in intestinal inflammation. Overexpression of PGHS-2 induces cellular oxidative stress. NO reacts with superoxide produce under oxidative stress to form peroxynitrite, a potent toxic oxidant. To investigate whether, PGHS-2 isolated from sheep placenta was treated with peroxynitrite and nitration of PGHS-2 was analyzed by Western blot analysis using antibodies against nitrotyrosine. Nitrotyrosine levels of PGHS-2 dramatically increased after treatment with 300μM peroxynitrite. Surprisingly, addition of 30μM GSH to the reaction mixture prevented nitration of PGHS-2 by peroxynitrite. This result suggests that GSH prevents peroxynitritemediated nitration of PGHS-2 by formation of nitrosoglutathione and that nitration of GSH futher increases oxidative stress by lowering cellular GSH levels. (Sponsored by NIEHS SBIR contract ES95438(HK)and the Petroleum Research Fund 33957-GB4 (ESR-K).

666 DIGESTIBILITY OF PROTEINS IN SIMULATED GASTRIC FLUID.

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Previous investigators have reported that 16 purified food protein allergens exhibited markedly greater stability in simulated gastric fluid (SGF) *in vitro* than 9 common plant proteins, and have concluded that stability in SGF distinguishes food allergens from nonallergens (Astwood et al., Nature Biotechnology 14: 1269, 1996). In the present study, the stability of 17 food protein allergens in SGF (0.08% (w/v) pepsin, 0.2% NaCl, 0.7% HCl) at 37°C has been compared with the stability of 24 proteins not associated with food allergy (17 plant food proteins and 7 animal proteins). Protein stability was assessed by SDS-polyacrylamide gel electrophoresis, using a gel system that resolved polypeptides with molecular masses that exceeded approximately 2 kDa. 13 food protein allergens were partially or completely stable for at least 60 min and/or were cleaved to polypeptide fragments that persisted for at least 60 min. 2 food allergens were degraded to peptides that were stable for 15 min and 2 were degraded to peptides that were stable for 5 min. Of the 17 plant food proteins that were tested, 8 were degraded completely within 5 min, as was 1 of the animal proteins. In contrast, 3 plant proteins resisted digestion for at least 60 min (e.g. potato tuber carboxypeptidase inhibitor), while 3 yielded polypeptide fragments that persisted for at least 60min (e.g. turnip cytochrome f). Peptide fragments that persisted for at least 60 min were also observed with 4 of the animal proteins (e.g. horse heart myoglobin). These results confirm that stability in SGF is a general feature of many food protein allergens. However, it is clear that similar stability is exhibited by other plant and animal proteins not suspected to be allergens. The implication is that resistance of food proteins to digestion in SGF is not a defining characteristic of allergenic potential.

667 SOY DIETS CONTAINING GENISTEIN STIMULATE GROWTH OF ESTROGEN-DEPENDENT TUMORS IN A DOSE DEPENDENT MANNER.

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We have demonstrated that genistein and the glycoside form, genistin, stimulate estrogen (E)-dependent human breast cancer (MCF-7) cells *in vivo* (Cancer Research 58:3833, FASEB J. 13(4) A370). Genistin which is metabolized to genistein after consumption is the predominant isoflavone in soy protein commercially available. Depending on processing, soy isolates can contain different concentrations of genistein. We hypothesize that soy protein

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