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Serum RIP3 level as a severity-predictive marker for Stevens-Johnson syndrome and toxic epidermal necrolysis

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Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening diseases. It is often difficult to distinguish SJS/TEN and other types of generalized skin eruptions, such as maculopapular exanthema (MPE) and erythema multiforme (EM) at the early stage. Although keratinocytes have been suggested to die as apoptosis in SJS/TEN, our recent study revealed that necroptosis, programmed necrosis, also contribute to keratinocyte death in SJS/TEN. Necroptosis is mediated by receptor-interacting kinase-3 (RIP3) and mixed lineage kinase domain-like pseudokinase phosphorylation. We aim to investigate whether serum RIP3 levels serve as a predictive biomarker for SJS/TEN severity. The serum sampled were obtained from the patients with SJS/TEN (n=13), EM major (n=19), EM minor (n=5) and MPE (n=6) in the acute phase. SJS/TEN group have significantly higher serum RIP3 levels than the other groups (EM major: P<0.002, EM minor: P< 0.003, MPE: P<0.003, healthy controls: P<0.002). Also in the EM major group, serum RIP3 levels are significantly higher than in the healthy controls (P<0.006). In addition, positive correlations were found between serum RIP3 levels and the frequency of keratinocyte death in histopathological examination, body temperature, mucosal involvement, and organ dysfunction. We also analyzed changes in serum RIP3 levels after initiation of treatment (SJS: n=4, EM: n=4) and after clinical recovery (SJS: n=3, EM: n=3). In all these samples, serum RIP3 levels decreased after treatment. We showed the clinical usefulness of serum RIP3 levels as a differential or prognostic marker for MPE, EM or SJS/TEN. By predicting of the severity at the early stage, we can start appropriate treatment earlier.

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Profile of BP180 and BP230-specific IgE autoantibodies in bullous pemphigoid

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Background: Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by autoantibodies against BP180 and BP230. There is emerging evidence that IgE autoantibodies play an important role in the pathogenesis of BP. Objective: To determine the rate of anti-BP180 and anti-BP230 IgE in BP, and to evaluate their diagnostic relevance in BP sera. Methods: We collected serum samples from 156 patients who met the clinical and immunologic, and histologic features consistent with BP. In addition, 10 control individuals were included for comparison. We used commercially available IgG enzyme-linked immunosorbent assay (ELISA) to develop IgG and IgE ELISAs for both BP180 and BP230. A receiver operating characteristic (ROC) curve was used to evaluate the ability of the ELISA to detect anti-BP180 and anti-BP230 IgE autoantibodies. Anti-BP180 IgE and anti-BP230 IgE positivity were conducted using the Spearman's rank correlation test. The results of IgE ELISAs were statistically compared among various ELISAs. Results: In 156 BP patients, 96 BP patients with elevated level of total IgE (>165.3IU/ml) and 60 BP patients with normal level of total IgE. IgE autoantibodies to BP180 and BP230 were found in 25 and 36 of 96 BP sera, respectively. And, anti-BP180 IgG and anti-BP230 IgG were detected in 82 and 79 of 96 BP sera, respectively. Anti-BP230 IgE autoantibodies, but not anti-BP180 IgE autoantibodies, correlated with total IgE (R=0.6914, P<0.0001). The level of BP230 IgE was significantly higher than the level of BP180 IgE (P=0.0357). Conclusion: The results of this study indicated that most BP patients exhibit elevated IgE levels in the serum. IgE autoantibodies to both BP180 and BP230 can be detected in BP sera. The results of total IgE and anti-BP230 IgE ELISAs are well correlated. In BP patients, the positivity rate of anti-BP230 IgE is higher than anti-BP180 IgE. IgE anti-BP230 autoantibodies seemed to be pathogenic in BP.

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Nanoparticle vaccine of cervical cancer based E7-HSP110 activate the exclusive killing-tumor response of CD8$^+$T cells in mice

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HPV16 E7 protein has been recognized as a target in the design of therapeutic vaccines for cervical cancer. However, many epitope vaccines have been limited in practical application due to design defects. It is still necessary to explore new vaccine forms to enhance its immunogenicity to HPV16 related tumors. In this study, we aimed to design anovel self-assembled nanoparticle HPV16 vaccine through combining RGD-GGG-$K_{18}$and fusion double expression plasmidpIRES2-3×E7-HSP110-EGFP (RGD-GGG-K_{18}/pIRES2-3×E7-HSP110-EGFP). The nanoparticles were prepared and identified by gel retardation, transmission electron microscopy and DNase I protection assays. Flow cytometry, *ex vivo*specific cytolysis of CTL-lactate dehydrogenase release analyzed the vaccine-induced immune responses. Immunization of TC-1 tumor-bearing mice with nanoparticles generated a larger pool of E7-specific CD8$^+$T cells with increased effector functions than the control groups. The *in vivo*tumor protective effectiveness of nanoparticle was investigated in the prophylactic and therapeutic mice models. Ultimately, the nanoparticles vaccine targeted for hpv16 E7 oncogene by significantly inhibited the growth of subcutaneous TC-1 tumor. The vaccine form designed in this study not only provides a potential choice for the development of new HPV vaccine, but also provides a new idea for specific immunotherapy of cervical cancer.

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Diet-induced obesity predisposes anti-PD-1 antibody-treated mice to imiquimod-mediated psoriasiform dermatitis: implications for immune-related adverse events in cancer patients treated with anti-PD-1

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Immune checkpoint inhibitors, including anti-PD-1, have led to tremendous progress in cancer treatment, but exacerbation of psoriasis or de novo development of psoriasis-like lesions occur in some cancer patients receiving anti-PD-1 treatment. Given that obesity is an exacerbating factor for psoriasis, we hypothesized that obese mice have more exacerbated imiquimod (IMQ)-induced psoriasiform dermatitis (PsD) than lean mice after administration of anti-PD-1. Obese mice were obtained by feeding C57BL/6 mice with a Western diet (WD) (moderate levels of fat and high levels of simple sugars) while lean mice were fed with a control diet (CD). After feeding for 12 weeks, WD mice weighed 30% more than CD-fed mice. Both groups underwent topical a 5-day IMQ treatment course to induce PsD. Compared with lean mice, obese mice had higher baseline expression of psoriasis-associated cytokines such as IL-17A (44-fold, P=0.007), IL-23 (2-fold, P=0.047), and S100A8 (39-fold, P=0.002) and higher PD-1 expression (48% vs. 26%, P=0.046) on γδ low T cells, which are major producers of IL-17A in murine PsD. After anti-PD-1 treatment, obese mice had an exaggerated skin response to IMQ compared to lean mice (ear thickness change on day 5: 94μm vs. 47μm, P<0.0001). Our results have clinical implications in that obesity may be a risk factor for exacerbation of psoriasis-like dermatitis during anti-PD-1 treatment. Furthermore, they provide a mechanistic basis for this known complication of checkpoint inhibitor therapy because of the greater baseline expression of PD-1 on IL-17A-producing, γδ-low T cells in WD-fed, obese mice.

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Global knockout of immunomodulatory indoleamine 2,3-dioxygenase has no effect on psoriasiform lesions in the imiquimod-induced mouse model of psoriasis

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Indoleamine 2,3-dioxygenase (IDO) is a cytosolic rate-limiting immunomodulatory enzyme that degrades tryptophan, resulting in tryptophan depletion and the accumulation of metabolites. The expression/activity of IDO in various cells, such as macrophages, dendritic cells, and trophoblasts, has been demonstrated to block T cell responses in a number of situations, including towards allogeneic fetuses and grafts/transplants. IDO induces immune suppression through two non−mutually exclusive mechanisms: (1) IDO degrades tryptophan into kynurenine, thus depleting the microenvironment of this essential amino acid and "starving" immune cells and (2) the kynurenine produced by IDO is actively immune suppressive through mechanisms including the induction of regulatory T cells (Tregs) through binding to the aryl hydrocarbon receptor. We hypothesized that IDO knockout mice would exhibit a hyperactive immune response because of decreased Treg function and therefore an exacerbation of the psoriasiform phenotype in the imiquimod-induced mouse model of psoriasis, an immune-mediated skin disease. Littermate wild-type and IDO knockout mice were treated with imiquimod, and ear edema, the severity of skin lesions, and the thickness of the epidermis were monitored. Unexpectedly, imiquimod-treated IDO knockout and wild-type mice exhibited a similar increase in ear edema, psoriasis area and severity index (PASI) scores, and epidermal thickness, indicating no effect of IDO gene loss on the skin's response to imiquimod. While these data may suggest a lack of involvement of IDO and Tregs in skin inflammation, other possible mechanisms, such as compensatory changes or the possibility that imiquimod already maximally suppressed Treg function, must also be considered.

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Differentiation of acute graft-versus-host disease from drug reaction in skin by a novel tissue-based biomarker assay

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Acute graft-versus-host disease (GVHD), a serious complication of allogeneic hematopoietic cell transplantation (HCT), often involves the skin. Since clinical and microscopic features of GVHD can mimic those of certain drug eruptions, accurate diagnosis can be challenging. Currently, no ancillary testing is available to improve diagnostic certainty. We aimed to develop a tissue-based molecular assay and clinical-molecular fusion model to improve differentiation of GVHD from drug eruptions following HCT. We queried our institution's HCT database (2010-2016) for skin biopsy specimens showing vacuolar interface changes attributable to GVHD or drug reaction, as classified by retrospective review of clinical records. Following biomarker discovery using unbiased molecular methods (i.e. proteomics, RNAseq) on archived skin biopsy specimens, we designed and tested a quantitative RT-PCR-based biomarker assay and generated clinical-molecular fusion diagnostic models. Of 62 putative tissue-based biomarkers, 26 were included in the assay. Significant differences in gene expression (*MX1, MNDA, OAS, LIMA1, GSTM5,* and *SPP1*) were detected between cases of GVHD (n=67) and post-HCT drug reaction with vacuolar interface changes (n=17; p<0.05). An optimal fusion model, incorporating quantitative expression of *MX1, MNDA, OAS,* and *GSTM5,* as well as presence of diarrhea, imparted high diagnostic accuracy (ROC AUC: 0.89). This novel tissue-based diagnostic clinical-molecular fusion model appeared to distinguish GVHD from drug eruptions in this retrospective cohort. Multicenter validation studies are required.