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Conversion of alloantigen-specific CD8+ T-cell anergy to CD8+ T-cell priming through in vivo ligation of glucocorticoid-induced TNF receptor

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In this study, we investigated the effect of an agonistic mAb (DTA-1) against GITR in a murine model of systemic lupus erythematosus-like chronic graft-versus-host disease (cGVHD). A single dose of DTA-1 inhibited the production of anti-DNA IgG1 autoantibody and the development of glomerulonephritis, typical symptoms of cGVHD. DTA-1-treated mice showed clinical and pathological signs of acute GVHD (aGVHD), such as lymphopenia, loss of body weight, increase of donor cell engraftment, and intestinal damage, indicating that DTA-1 shifted cGVHD towards aGVHD. The conversion of cGVHD to aGVHD occurred because DTA-1 prevented donor CD8+ T-cell anergy. Functionally active donor CD8+ T cells produced high levels of IFN-ɣ and had an elevated CTL activity against host Ags. In in vitro MLR, anergic responder CD8+ T cells were generated and DTA-1 stimulated the activation of these anergic CD8+ T cells. We further confirmed in vivo that donor CD8+ T cells, but not donor CD4+ T cells, were responsible for the DTA-1-mediated conversion of cGVHD to aGVHD. These results indicate that donor CD8+ T-cell anergy is a restriction factor in the development of aGVHD and that in vivo ligation of GITR prevents CD8+ T-cell anergy by activating donor CD8+ T cells that otherwise become anergic.

H-14

CpG-ODNs restore UVB-induced immunosuppression of contact hypersensitivity in mice

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UV radiation plays a critical role in immune suppression to generate antigen-specific T cells, which inhibit cell-mediated responses like contact hypersensitivity (CHS) reactions. Synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG dinucleotide are known to have the immunostimulatory activities in mice and to convert from Th2 to Th1 immune responses in atopic disease. We aimed to investigate the effects of CpG-ODNs on UVB-induced immunosuppression of CHS in mice. Female Balb/c mice were exposed to UVB and CpG-ODNs were injected into the mice via i.p. or i.v. and at different time after UVB-irradiation. Then, the mice were sensitized and challenged. The efficacy of CpG-ODNs was measured by ear swelling and histopathological examinations. Both i.p. and i.v. injection of the CpG-ODNs resulted in the significant recovery of the CHS controlled by UVB. Histopathologically, UVB-irradiated and CpG-ODN injected mice showed pronounced inflammatory cell infiltration compared with UVB-irradiated and CpG-ODN non-injected mice. UVB-irradiated and CpG-ODN treated mice showed the same degree of ear swelling compared with only TNCB-sensitized mice. Taken together, CpG-ODNs might restore the UVB-induced immunosuppression and be applied in preventing UVB-induced harmful effects.

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CpG-Oligodeoxynucleotide- and lipopolysaccharide-induced NF-κB activation control Schlafen-2 gene transcription in macrophage cell line RAW 264.7

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To screen LPS- or CpG-ODN-responsive inflammation related gene expressions, we examined the differential gene expressions in LPS or CpG-ODN-stimulated RAW 264.7 cells. Using differentially displayed PCR method, we identified Schlafen-2 (Slfn-2) as potential LPS- or CpG-ODN-responsive element in RAW 264.7 cells. A putative Slfn-2 promoter-reporter was activated by ectopical expression of NF-κB p65 transcription factor. Inhibition of NF-κB p65 activation by treatment of BMS-345541, an IKK-2 inhibitor, or overexpression of a mutant IκBα protein blocked LPS- and CpG-ODN-induced Slfn-2 transcription, showing that NF-κB activation is required for Slfn-2 gene expression in the LPS- or CpG-ODN-signaling pathway. Furthermore, chromatin immunoprecipitation and site-specific mutant analysis on NF-κB binding site confirmed that NF-κB was important to regulate LPS- and CpG-ODN-induced Slfn-2 gene expression in RAW 264.7 cells. In summary, our results indicate that LPS-induced and CpG-ODN-induced NF-κB activation play a pivotal role in controlling gene expression of Slfn-2 in macrophage cell line RAW 264.7. [This work was supported by Korea Research Foundation Grant funded by Korea Government, Basic Research Promotion Fund, KRF-2005-070-C00091]

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Crea-10 and related cytokines are likely to involve in the FLS hyperproliferation in the pathogenesis of Rheumatoid arthritis

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Rheumatoid arthritis (RA) is an inflammatory autoimmune disease of unclear etiology. This study was initiated to identify more critical factors involved in the mechanisms by which synovial hyperplasia occurs in RA. We applied cDNA microarray analysis to profile gene expressions of RA fibroblast-Like synoviocytes (FLSs) from 3 RA patients or bone marrow dendritic cells (BmDCs) from DBA/1 mice. We found that the Crea-10 gene known to be mainly overexpressed in breast cancer is remarkably up-regulated in the cells related to RA. In addition, GM-CSF in all synovial fluids (SFs) from RA patients (n = 6) revealed to evenly exist to a significant level, compared to other inflammatory cytokines, IL-1β and TNF-α. Most FLSs in passage 10 or more recovered from growth retardation when cultured in the presence of 1/10-diluted SF. The SF-mediated growth recovery was markedly blocked by anti-GM-CSF neutralizing antibody. Growth-retarded RA-FLS recovered their proliferation capacity by GM-CSF at a concentration of 100 ng/ml. These results imply that GM-CSF in SF plays an important role for the hyperproliferation of RA FLS. In the microarray analysis and semi-quantitative RT-PCR, we found that the Crea-10 was highly expressed in the hyperactive RA FLS in low passage or RA FLS cultured in the presence of SF or GM-CSF. Moreover Crea-10 expression was enhanced by GM-CSF to a significant level, and FLS proliferation also was improved by GM-CSF treatment at a dose-dependent manner. Crea-10 knock-down by siRNA completely blocked the GM-CSF/SF-mediated hyperproliferation of RA FLS, suggesting that the Crea-10 is strongly involved in the chronic synovitis in RA patients in line with GM-CSF.