tional ASPCR was either non-specific at all or showed specificity only in a very stringent conditions, which included low concentration of primers and magnesium chloride, high annealing temperature, and low number of PCR cycles; when any of the mentioned parameters was even slightly relaxed, non-correct genotyping occurred. However in the presence of the 3-fold excess of the depository oligonucleotide, ASPCR retained the specificity and reproducibility even if the PCR stringency was significantly reduced.

Conclusions: The deposition of allele-specific primers by complementary oligonucleotides evidently increased the reliability of ASPCR. The proposed modification may substantially facilitate SNP genotyping, either alone or in combination with other ASPCR improvements.

606 POSTER

Streptococcal preparation OK-432 is a new GMP-grade maturation factor of monocyte-derived dendritic cells

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Background: For vaccinations based on dendritic cells (DCs), maturation of DCs is important for the induction of effective T cell responses. A streptococcal preparation, OK-432, has been used as multi-cytokine inducer for management of cancer patients in Japan. We examined whether OK-432 can be a Good Manufacturing Practice (GMP)-grade maturation factor of DCs.

Material and methods: Immature monocyte-derived DCs (imDCs) generated from human peripheral blood mononuclear cells with granulocyte-macrophage colony stimulating factor and interleukin (IL)-4 were exposed to two types of common maturation factors, i.e., lipopolysaccharide and tumor necrosis factor-alpha plus prostaglandin E2, or OK-432 for another 2 days. Their surface expression of maturation-related molecules, allogeneic T cell proliferation, and cytokine secretion were analyzed with fluorescence-activated cell sorting (FACS), allogeneic mixed-lymphocyte reaction, and enzyme-linked immunosorbent assay, respectively. Activation of nuclear factor kappa B (NF- kappa B) was also examined with electrophoretic mobility shift assay.

Results: All agents examined increased both expression of maturation-related molecules such as HLA-DR, CD80, CD83, and CD86, and allogeneic T cell proliferation at a similar level in imDCs. Importantly, only OK-432 caused significant production of IL-12 p70 and interferon-gamma (IFN-gamma) at both the mRNA and protein levels. Induction of intracellular IL-12 and IFN- gamma in OK-432-stimulated DCs was also confirmed with FACS Calibur. Moreover, OK-432 induced activation of NF- kappa B in imDCs. Both cytokine secretion and NF- kappa B activation induced with OK-432 were suppressed when imDCs were pretreated with cytochalasin B, an inhibitor of endocytosis.

Conclusion: Our experimental data indicate that uptake of OK-432 by imDCs is an early critical event for secretion of both IL-12 p70 and IFN- gamma and that activation of NF- kappa B induced by OK-432 also contributes partially to these cytokine secretion. Since OK-432 is a GMP-grade agent, OK-432 may be a potential tool for vaccinations based on DCs.

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Induction of cytotoxic T lymphocytes that recognize a tumor-associated antigen, 90K/Mac-2 binding protein with an HLA-A2 restriction

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Background: 90K/Mac-2 binding protein (M2BP) is highly expressed in patients with various types of cancer and can influence the expression of surface molecules involved in immune responses on cultured cancer cells. We have reported that M2BP-specific immunity was observed in many lung cancer patients (Cancer 2002; 95: 1954-62). In this study, to identify HLA-A2-restricted immunogenic epitopes of M2BP, we generate cytotoxic T Lymphocytes specific for M2BP in vitro.

Materials & Methods: We selected 11 peptides (9-mer or 10-mer) derived from M2BP with an HLA-A*0201 binding motif according to peptide-motif scoring algorithms. M2BP-specific CTLs were generated from peripheral blood lymphocytes (PBLs) of HLA-A2-positive healthy donors by multiple stimulations of CD8-positive T lymphocytes with M2BP peptides. The induced CTL lines were examined for their specific responses to antigens by interferon-gamma production and standard ^{51}chromium-release assays.

Results: Three of the 11 CTL lines produced interferon-gamma in response to T2 cells (M2BP-/HLA-A2+) pulsed with the same peptide with a dose-dependent manner. However, only one CTL line induced using M2BP216-224 could lyse both peptide pulsed-T2 cells and a breast cancer cell line, MDA-MB-231 cells (M2BP+/HLA-A2+). The cytolysis was blocked by antibodies against HLA class I but not HLA class II molecules.

Conclusion: M2BP-specific CTLs could be generated in vitro using M2BP216-224 peptide. M2BP is expected to be useful as a target antigen in cancer immunotherapy.

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Streptococcal preparation ok-432 induces human dendritic cells maturation via up-regulation of toll-like receptors

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Dendritic cells (DCs) are potent antigen presenting cells to promote specific anti-tumor immune response. Streptococcal preparation OK-432 is supposed to induce innate immunity and up-regulation of toll-like receptors (TLRs). In the present study, we have investigated the effect of OK-432 on expression of TLRs as well as on maturation and activation of DCs in comparison with conventional tumor necrosis factor (TNF)-alpha. Human peripheral blood mononuclear cells (PBMC) were collected from five healthy volunteers and cultured in serum-free medium (AIM-V) in the presence of interleukin-4 (IL-4; 50ng/ml) and granulocyte-macrophage stimulating factor (GM-CSF; 50ng/ml) for 6 days. Then DCs were pulsed with tumor cell lysate obtained from human gastric cancer cell line MKN-45 for 12hr and further cultured for 48hr following addition of OK-432 (0.1 KE/ml). We compared it with addition of TNF-alpha (100 ng/ml) for DCs maturation. Cell surface phenotypes of DCs (HLA-ABC, HLA-DR, CD40, CD54, CD80, CD83 and CD86) were examined by flow cytometry, and cytotoxic T cell activity was evaluated using ^{51}Cr releasing assay. Expression of toll-like receptor (TLR)-4 and TLR9 after stimulation by OK-432, TNF-alpha or lipopolysaccharide (LPS) were examined using real-time reverse transcription polymerase chain reaction (RT-PCR). Expression of cell surface phenotypes examined was increased either on the surface of TNF or OK-432 treated DCs in a time dependent manner. No significant difference of the intensity of expression was noted between the two groups. Furthermore, ^{51}Cr releasing assay showed specific cytotoxity for MKN-45 with similar killing activity between the two groups. Expression of TLR-4 and TLR-9 were highest after LPS treatment, followed by OK-432 and TNF treatment, significantly higher in OK-432 treated group than in TNF treated group. The expression of TLRs peaked at 1 hr after stimulation in LPS and TNF, while it peaked at 2 hr after stimulation in OK-432. These results suggest that OK-432 has a potential role on human DCs for generation of CTL possibly via up-regulation of TLRs, and would offer an eligible protocol for human DCs in vivo immunotherapy especially for local administration.

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Tumour burden and interleukin-2 dose affect the synergism between low-dose total body irradiation and interleukin-2

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Background: Low-dose total body irradiation (LTBI) is believed to initiate various immune-mediated anti-tumour effects. We have previously shown a synergistic therapeutic effects when LTBI was used in combination with Interleukin-2 (IL-2) in a murine metastatic malignant melanoma model.

Aim of the work: To optimise the use of this combination treatment this study was performed to test the effect of tumour burden and dose of both LTBI and IL-2 on the therapeutic potential of this treatment strategy.

Material and Methods: Ten-week-old female C57BL/6 mice were inoculated i.v. (Day 0) with 1 million B16F1 malignant melanoma cells. The mice received either: no treatment, single fraction of LTBI alone, IL-2 treatment alone, or a combination of LTBI and IL-2. Two dose levels of LTBI and IL-2 were tested. LTBI was given either on day +7 or on day +10. IL-2 treatment was given over 5 days starting 24 hours after LTBI. Two days after the end of treatment, the mice were sacrificed and the lungs were removed and analyzed for tumor burden. Lung sections were also tested for tumor infiltrating cells using immuno-histochemical staining.

Results: LTBI (in the 2 tested dose levels), showed to independent therapeutic effects. IL-2 dose of (300.000 CU) that proved effective and