Laboratory of Tumour Immunology

Anti-tumour immunotherapy, immunoediting, immunoepigenetics, NKT cells, immune suppression

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As a long-term research programme of our laboratory we have been investigating the mechanisms by which tumour cells are capable to escape from immune responses. Our projects are focused on regulation of genes encoding molecules crucial for antigen presentation and for tumour cell elimination by specific immune responses, on mechanisms of immunosuppression and its possible overcoming, and, finally, on experimental anti-tumour immunotherapy. Most of our studies employ murine models for tumours associated with human papilloma virus (aetiological agent of the cervical carcinoma). Special attention has been paid to the setting of the minimal residual tumour disease treatment after primary tumour resection or chemotherapy.

MHC class I deficiency on tumour cells is a frequent mechanisms by which tumour cells can escape from specific immune responses. We have been interested in mechanisms underlying reversible MHC class I downregulation on tumour cells. We have found that epigenetic agents induce expression of genes involved in antigen-processing machinery and surface expression of MHC class I molecules on tumour cells, as well as of selected co-stimulatory and co-inhibitory molecules. Further analysis revealed that MHC class I downregulation in our model cell lines was associated with epigenetic silencing of antigen-presenting machinery genes. Our current projects in this field are focused on *in vivo* experiments with the aim to optimize immunotherapy of MHC class I-deficient tumours combined with administration of DNA methyltransferase inhibitors. We are also interested in epigenetic mechanisms underlying regulation of genes encoding antigen-presentation machinery genes, as well as co-stimulatory/inhibitory genes in antigen-presenting or regulatory immunocytes.

Our next areas of interest are populations of immunoregulatory cells (NKT, T-regulatory and myeloid-derived suppressor cells) and their dynamics in the course of tumour growth and therapy, as well as their mutual interactions. Recently, we have shown that administration of a glycolipid ligand activating NKT cells, β-galactosylceramide, inhibited growth of recurrent MHC class I-positive and -deficient tumours after surgery.

Along with these projects we have been interested in experimental anti-tumour immunotherapy and vaccines. We have been investigating the impacts of several chemotherapeutic agents on anti-tumour immunity and on regulatory cell populations. We have used cell and gene therapy approaches and dendritic cell-based vaccines, as well as genetically modified tumour cells producing cytokines (especially IL-12-producing cells) for vaccination and immunotherapy optimization.

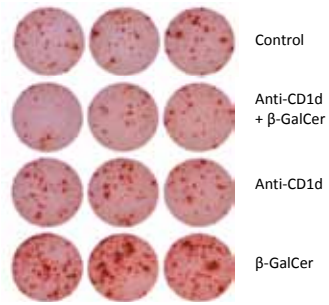

Fig. 1. ELISPOT (Enzyme-linked immunosorbent spot) analysis of the spleen cell activation with a ligand for NKT T-cell receptor (C12 β-D-GalactosylCeramide) and its inhibition with the anti-CD1d-antibody demonstrates that the C12 β-D-GalactosylCeramide effects are mediated through its presentation by the CD1d molecules. Each colour spot represents one interferon γ-producing spleen cell.

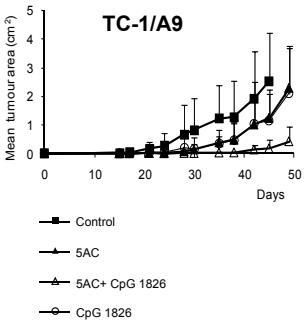

Fig. 2. Tumour growth curves in control mice and mice subjected to chemo- and immunotherapy and their combination. Tumour inhibitory effects of combined epigenetic agents, 5-azacytidine (5AC) and immune activator unmethylated oligodeoxynucleotide (CpG 1826). MHC class I-deficient tumour cells were transplanted on day 0. In experimental groups, 5AC was repeatedly administered on days 3, 7, 10, 14, 17, 21, 24, 28; immune activator, CpG 1826 was administered on days 3 and 10. Significant inhibition was observed in all treated mice, as compared to the untreated controls. Combined therapy was significantly more effective as compared to monotherapies only.

- FP6 EU, 18933 CLINIGENE – European network for the advancement of clinical gene transfer and therapy, 2006-2011, J. Bubeník
- AS CR, IAA500520605 – Induction of immunity against HPV16 E6/E7 oncogenes-induced tumours with peptide- and dendritic cell-based vaccines; immunotherapy of minimal residual tumour disease, 2006-2009, M. Reiniš
- GA CR, GA301/07/1410 – Immunosuppressive cell populations in the course of tumour progression and therapy, 2007-2011, M. Reiniš
- AS CR, IAA500520807 – Mechanisms of protective immunity against tumours with different expression of immunodulatory molecules, 2008-2010, M. Indrová
- Ministry of Health of the Czech Republic, NS10660 – Development of experimental anti-WT 1 vaccine for immunotherapy of tumours, 2009-2011, M. Indrová
- GA CR, GA301/09/1024 – Molecular and cellular mechanisms of tumour chemotherapy: immunomodulatory effect, 2009-2011, M. Indrová
- GA CR, GAP301/10/2174 – Epigenetic mechanisms in regulation of genes important for antigen presentation and antitumour immunity, 2010-2013, M. Reiniš

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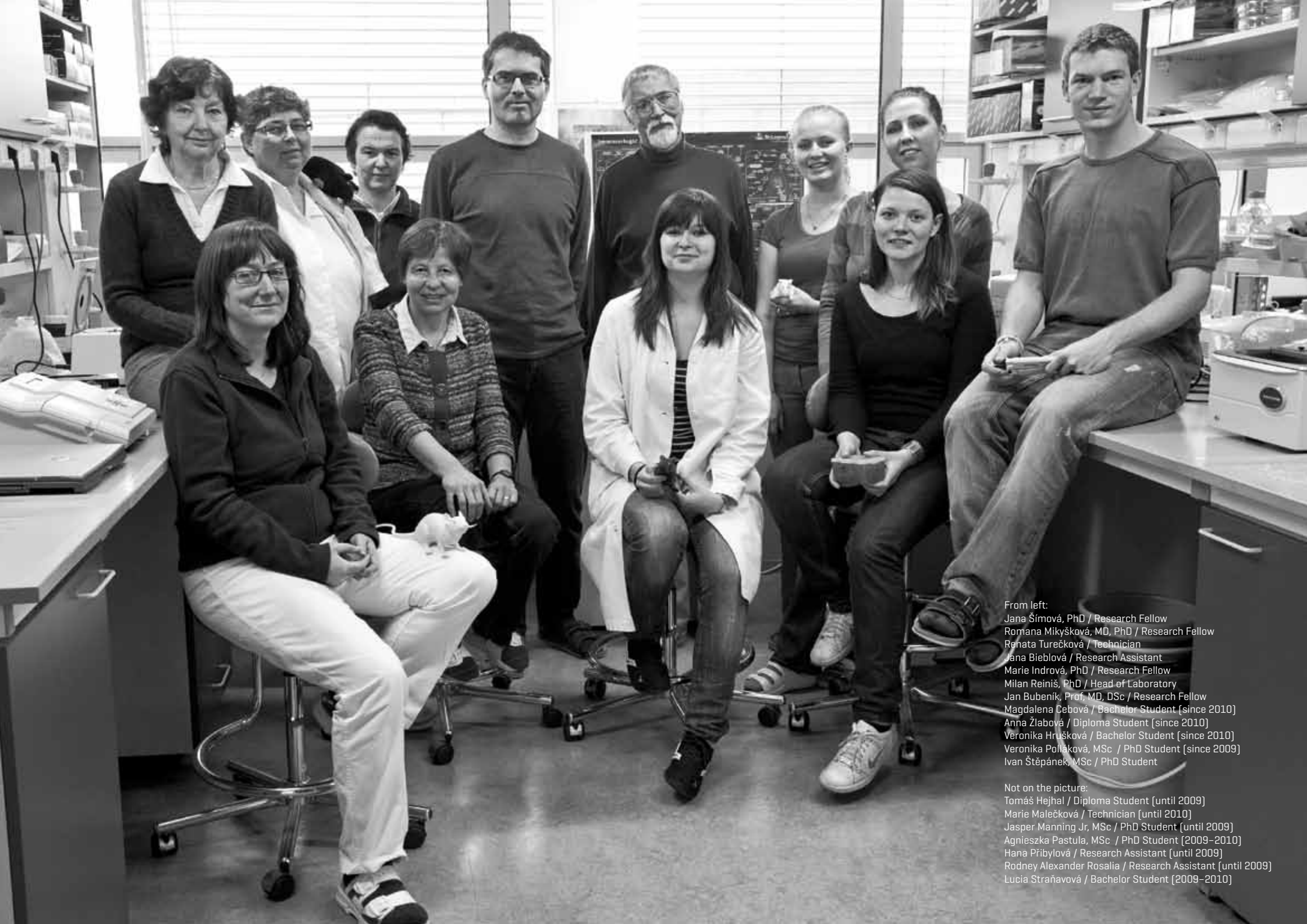
2. Šímová J, Indrová M, Bieblová J, Mikyšková R, Bubeník J, Reiniš M. Therapy for minimal residual tumor disease: β-Galactosylceramide inhibits the growth of recurrent HPV16-associated neoplasms after surgery and chemotherapy. **Int J Cancer 2010** 126(12): 2997-3004.
3. Indrová M, Bieblová J, Rossowska J, Kuropka P, Pajtasz-Piasecka E, Bubeník J, Reinis M. HPV 16-associated tumours: IL-12 can repair the absence of cytotoxic and proliferative responses of tumour infiltrating cells after chemotherapy. **Int J Oncol 2009** 34(1): 173-179.
4. Bubenik J. Genetically modified cellular vaccines for therapy of human papilloma virus type 16 (HPV 16)-associated tumours. **Curr Cancer Drug Targets 2008** 8(3): 180-186.
5. Manning J, Indrová M, Lubyová B, Přibylová H, Bieblová J, Hejnar J, Šímová J, Jandlová T, Bubeník J, Reiniš M. Induction of MHC class I molecule cell surface expression and epigenetic activation of antigen-processing machinery components in a murine model for human papilloma virus 16-associated tumours. **Immunology 2008** 123(2): 218-227.



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