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STRESS-RELATED ADAPTATION CHANGES IN THE SPLEEN DURING EARLY POSTNATAL DEVELOPMENT

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Interconnections between the integrative systems, such as nervous, endocrine, and immune ones, are clearly seen during a stress response. The sources of such a cooperation should be sought at the earliest stages of development. Early negative life events, especially during the neonatal period, resulted in long lasting, irreversible effects on well being. Neonatal stress has implications for host resistance to infection throughout life. Thus, long lasting effects of negative life events on health and disease may be the basis for the individual differences in host susceptibility to infection, malignancy and autoimmune disorders. Age-related aspects of the reduced immunity following stress exposure in terms of possible mechanisms of their development remain not fully understood (**I.G.Akmaev et al., 2002; S.K.Butcher et al., 2005; R.Avitsur et al., 2006).**

The objective of this study was to compare immunomodulatory changes in the spleen as a peripheral organ of immune defense in different age groups of the growing experimental animals under the chronic effect of a severe stressor.

Thirty two Sprague-Dawley rats of the two age groups, each of which included 16 animals, were either exposed to the severe chronic (restraint) stress (**R.Kvetnansky et al., 1970**) with 7 daily 5-hour sessions (eight animals per subgroup) or used as an age-matched control (eight animals per subgroup). The 1st age group contained weaning animals aged 21 days and the 2nd age group included early postweaning animals age 30 days.

After the last session of stress the animals were weighed and euthanized by cervical dislocation. The lymphoid organs (thymus, spleen and mesenterial lymph nodes) were collected, weighed and processed for histological examination. Formalin-fixed paraffin sections were stained with haematoxylin-eosin and immunohistochemically stained by monoclonal antibodies (Serotek, UK) against rat CD8 (T-suppressor/cytotoxic lymphocytes), CD20 (B-lymphocytes) and CD68 (macrophages) surface markers using ABC-method (**J.Polack, 2000)** with subsequent image analysis of the profiles of the immunoreactive cells on the NIKON camera-captured digital pictures using Image Pro Plus 4.5 software.

At the end of the last stress sessions the body mass of the experimental animals was significantly reduced in both age groups ($p<0,05$). Relative splenic mass was also decreased in the experimental animals compared to the age-matched control groups in the weaning and early postweaning pups ($p<0,01$).

The results of the microscopic investigation presented dramatic immunomodulatory changes in different compartments of the spleen which were mainly localized in the splenic white pulp, with red pulp and marginal zones being also involved. The lymphatic follicles of the experimental rats of both age groups were reduced in number and size, lacked germinal centers and were filled with tingable-body macrophages containing numerous apoptotic bodies. Periarterial lymphoid sheaths also decreased in size mainly at the expense of their inner zone. Tingable body macrophages filled with apoptotic bodies were less common for the periarterial lymphoid sheathes compared to the lymphoid nodules. The marginal zone of the lymphoid nodules and periarterial lymphoid sheathes was reduced in width while this reduction was more prominent in the animals of the weaning experimental group.

The immunohistochemical staining for the CD8 of the control animals spleen exhibited immunoreactive cells localized mainly in the periarterial lymphoid sheathes with fewer cells in the red pulp, marginal zone and mantle zone of the lymphoid follicles. The accumulation of the CD8+cells increased with age. After chronic exposure to the severe stressor the number of immunoreactive cells in the periarterial lymphoid sheathes was notably reduced with single immunopositive cells still present in the red pulp and marginal zone. Staining for CD20 revealed concentration of the immunopositive cells in the lymphoid nodules with less dense distribution of the immunoreactive cells in the marginal zone and red pulp. After the last stress session the number of the immunoreactive cells appeared to be reduced in the splenic B-zones of the experimental animals of both age groups. Staining for CD68 demonstrated crowding of the immunopositive cells in the red pulp of the experimental and control animals of both age groups. In the lymphoid nodules and the marginal zones of the control rats they were very rare and in the splenic T-zones they were absent. In the experimental animals single immunopositive cells were also visible in the splenic T-zones.

Quantitative immunohistochemical analysis showed that the volume and numeric density of the CD8+ lymphocytes was significantly reduced in the weaning and early postweaning ($p<0.001$) experimental rats against the control groups of animals. The volume and numeric density of the CD20+ lymphocytes was meaningfully decreased in the weaning ($p<0.001$) and postweaning ($p<0.05$) experimental animals accordingly. The changes in the volume density of the CD68+ cells in the experimental animals did not reach the level of significance in both age groups compared to the control rats.

The results obtained demonstrated the presence of the immunosuppressive changes in the T- and B-zones of the splenic white pulp in the growing body which were most pronounced in the weaning period of the early postnatal development.

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SINGLE INTRATUMOURAL INTERLEUKIN-2 TREATMENT *PRIOR* SURGERY: LOCAL AND SYSTEMIC EFFECTS ON TRANSPLANTABLE MOUSE MAMMARY ADENOCARCINOMAS

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The problem of local and systemic breast cancer recurrence after radical mastectomy in both medical and veterinary practice is not yet solved. Earlier we showed in a number of mouse models of breast cancer that neither mammary carcinoma extirpation alone nor local *peritumoural* (PT) interleukin-2 (IL-2) therapy did not prevent deaths of recipient mice from tumour growth/relapse and/or lung metastases. However, advanced transplanted mouse mammary adenocarcinomas (MACs, therapeutic models) with early appearing tumours were more sensitive to single PT IL-2 therapy than late appearing MACs. It was also shown that single *intratumoral* (IT) IL-2 injection caused systemic anti-cancer effect in DBA/2 mice bearing advanced SL-2 transplanted lymphomas. We hypothesised that IL-2 therapy *prior* surgery may cause both local and systemic effects in preventing of appearance and growth of recurrent MACs and/or metastatic disease. To this end a combined surgical mouse model with a single *intratumoural* IL-2 application one week before MAC extirpation was developed. Growth rate of *contra lateral* untreated and non-extirpated "marker" MAC was used to visualise the IL-2 systemic effects. The aim of this research was to compare local and/or systemic effects of a single IT IL-2 application *prior* surgery in early and late arising transplantable MAC models. Local IL-2 effect was assessed before surgery by right-sided tumour growth delay and after surgery by frequency of appearance and growth rate of recurrent tumours. Systemic IL-2 effect was estimated observing the behaviour of untreated non-extirpated "marker" tumour and metastasis incidences. Efficacy of the IL-2 treatment was evaluated by the improvement in mouse survival in IL-2 treated groups *versus* controls. Surgery was provided under non-inhalation zoletil/vetranquil anaesthesia. All the recipients with advanced MAC loosing body weight were euthanized by cervical dislocation; tumours and organs of possible metastasis were analysed by gross morphology during *post mortem* examination and by histopathology.

Transplantable BALB/c MAC (Bc-MAC) in syngeneic males was used as an early appearing model having short latent period (lag<2weeks). Bc-MAC was previously characterised in female recipients as a fast growing non-metastasising MAC. Here, syngeneic males (n=33) were injected with 10^6 tumour cells (TC) *per* mouse at the right axillary fat pad (AFP) at day 0. At day 6 *post* transplantation (*pt*) all the recipients were additionally injected with 10^6 TC *per* mouse at the left AFP to produce a "marker" tumour. At day 10 *pt* single $1x10^6$ IU dose of IL-2 per mouse was applied IT to the right-sided MAC of an average size of 4.27±0.08mm (n=16). Control males bearing right-sided MAC of average size of 4.26±0.07mm (n=17) received physiologic solution in the same manner. At day 15 *pt* all treated right-sided MAC were surgically extirpated; almost all left sided "marker" tumours were palpable at this time.

Transplantable Wnt-1 induced MAC in C57BL/6 (B6-MAC, transplanted from original spontaneous transgenic MAC) females was used as a late appearing model having long latent period (lag>2w) of tumour growth and metastasis to the lungs in 67% of cases. Syngeneic B6 females (n=27) were injected with 10^6 B6-MAC TC *per* mouse at the right AFP at day 0. At day 9 *pt* all the recipients were additionally injected with 10^6 B6-MAC TC *per* mouse at the left AFP to produce a "marker" tumour. At day 29 *pt* the first subgroup of right-sided MAC (n=16) having latent period less than 4 weeks ($lag^{<4w}$) reached visible size and were selected for the IL-2 therapy; the second subgroup (n=11) still had palpable or none right-sided MAC at this time (long latent period, $lag^{>4w}$). Right-sided $lag^{<4w}$ MAC (6.1±0.8mm, n=8) were treated IT by a single $1x10^6$ IU IL-2 *per* mouse at day 29 *pt*. Control $lag^{<4w}$ MAC (5.3±0.9mm, n=8) received physiologic solution in the same manner. At day 36 *pt* all treated $lag^{<4w}$ right-sided MAC were surgically extirpated; none of the left-sided "marker" tumour was visible. Only at day 42 *pt* $lag^{>4w}$ right-sided MAC reached visible size (5.7±0.4mm, n=7) and were treated IT by a single $1x10^6$ IU IL-2 *per* mouse; control $lag^{>4w}$ right-sided MAC (6.0±0.3mm, n=4) received physiologic solution in the same manner. At day 49 *pt* all $lag^{>4w}$ right-sided MAC were surgically extirpated; none of the left sided "marker" tumour was visible at this time.

All the recipients with advanced MAC loosing tumour and body weight were euthanized by cervical dislocation; tumours and organs of metastatic potential were analysed by gross morphology during *post mortem* examination and by histopathology. Local IL-2 effect was assessed before surgery by right-sided tu-