Role of Mast Cells in Pneumococcal Meningitis

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Background: Pneumococcal infection of the leptomeninges generates a powerful inflammatory reaction which contributes essentially to meningitis-associated brain damage. There is still uncertainty about the cellular initiators of this hyper-inflammatory response. The leptomeninges are densely populated with mast cells (MC). *In vitro*, a human MC line has been shown to respond to pneumococcal challenge by degranulation (Barbuti et a., *Int.J Med.Microbiol.* 2006). Thus, we hypothesized involvement of MC in pneumococcal meningitis (PM).

Figure 1: Facs analysis of surface marker expression (CD117 and FcεR1) on wild type MC 4, 5 and 6 weeks after the start of culture

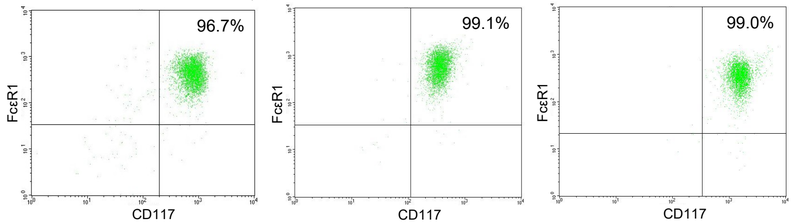
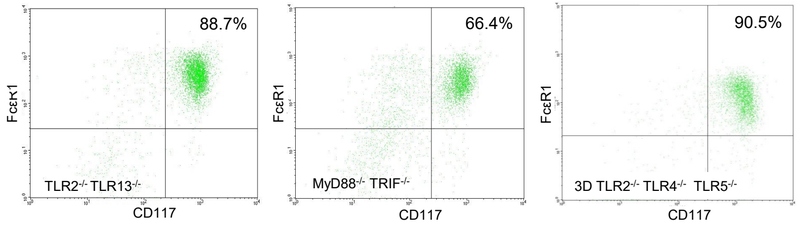


Figure 2: Facs analysis of surface marker expression (CD117 and FcεR) on MC from TLR2$^{-/-}$ TLR13$^{-/-}$, MyD88$^{-/-}$ TRIF$^{-/-}$ and 3D TLR2$^{-/-}$ TLR4$^{-/-}$ TLR5$^{-/-}$ mice 4 weeks after the start of culture



Material & Methods Murine MC derived from bone marrow cells of wild type (wt) -, MyD88/TRIF-, 3d-TLR2/4-, TLR2/13- and ASC -knockout mice were exposed to different serotypes of *Streptococcus (S.) pneumoniae* and assessed for degranulation by measuring the release of β-hexosaminidase and for cytokine production by ELISA. In supplemental experimental series, wild type MC were challenged with *S. pneumoniae* and concomitantly treated with diverse pharmacologic antagonists such as antibodies directed against complement receptor 1/2 or 3. Moreover, the phenotype (like cerebrospinal fluid (CSF) leukocyte counts and clinical score values) of two MC-deficient mouse strains (namely Kit^{W}/Kit^{W-v} and Kit^{W-sh}/$^{W-sh}$ mice) was evaluated in an established meningitis model.

Results: • **Murine bone marrow-derived mast cells (MC)** degranulated upon stimulation with *S. pneumoniae* and showed increased cytokine/chemokine production.

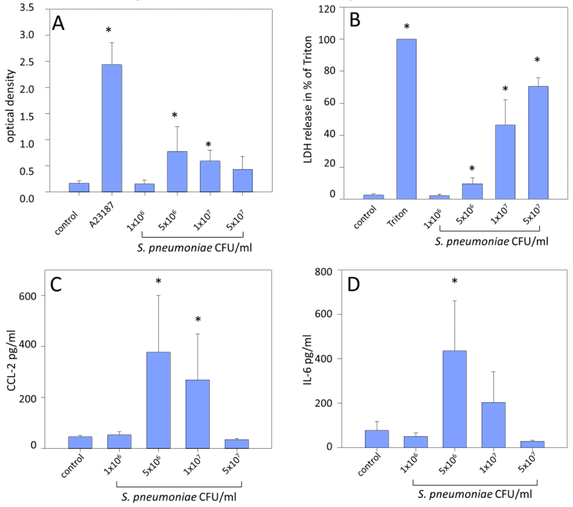


Figure 3: MC were stimulated with increasing concentrations of lysed *S. pneumoniae*. MC showed an increased release of β-hexosaminidase (a marker for degranulation, A), LDH (a marker for cytotoxicity, B), CCL-2 (C) and IL-6 (D). Experiments were done thrice in duplicates. CFU = colony forming units. Data are means ± SD. * p<0.05 compared to control, using ANOVA and Student-Newman-Keuls posthoc test.

• Pneumococci-induced MC activation was dependent on the presence of PLY and varied between different serotypes.

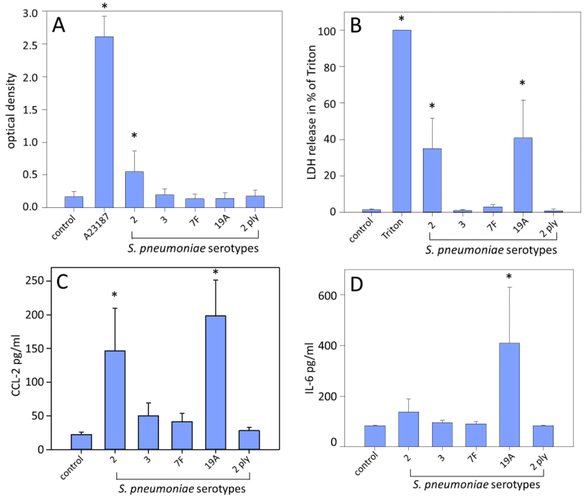
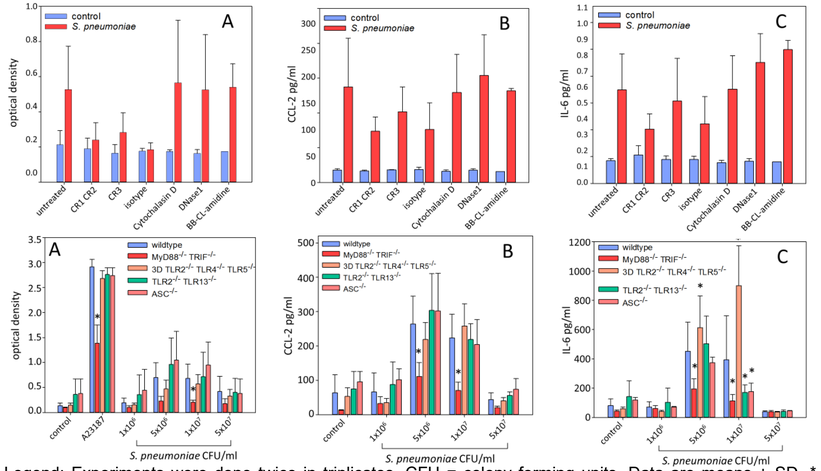


Figure 4: MC were stimulated with different *S. pneumoniae* serotypes (1x10^{7} cfu/ml each). MC degranulation (A), death (B), and cytokine production (C, D) were dependent on pneumolysin and varied between serotypes. Experiments were done thrice in duplicates. CFU = colony forming units. Data are means ± SD. * p<0.05 compared to control, using ANOVA and Student-Newman-Keuls posthoc test.

• MC activation occurs independent on MyD88/TRIF-TLR-signaling, complement activation, phagocytosis, and netosis.

Figure 5: Effect of pharmacologic inhibition of complement receptors, phagocytosis and netosis on MC degranulation (A), CCL-2 (B) and IL-6 (C) production.



Legend: Experiments were done twice in triplicates. CFU = colony forming units. Data are means ± SD. * p<0.05 compared to control, using ANOVA and Student-Newman-Keuls posthoc test.

• **In the meningitis model**, MC deficient mouse strains had higher CSF leukocyte counts, as compared to wt strains. MC reconstitution resulted in a partial reversal of this effect. However, there were no significant differences between infected wt and MC-deficient mouse strains in all other parameters assessed.

Effect of mast cell deficiency on inflammation, bacterial outgrowth and the clinical status in experimental pneumococcal meningitis

	N	CSF leukocyte counts [x 1000 cells/µl]	CSF IL-1b level [pg/ml]	Cerebellar bacterial titres [log$_{10}$ cfu/organ]	Blood bacterial titres [log$_{10}$ cfu/ml]	Clinical score
PBS-injected control	10	0.2 ± 0.1	< detection limit	< detection limit	< detection limit	0.0 ± 0.0
Infected WBB6F1-Kit$^{+/+}$	12	8.5 ± 3.2 *#	4,174 ± 3,287 *	6.7 ± 0.6 *	5.3 ± 0.3 *	7.0 ± 1.8 *
Infected Kit^{W}/Kit^{W-v} (MC deficient)	12	22.3 ± 7.4 *	2,760 ± 2,734 *	6.3 ± 0.5 *	5.4 ± 1.0 *	5.5 ± 2.0 *
Infected Kit^{W}/Kit^{W-v}, reconstituted with MC	6	11.9 ± 4.1 *#	not done	6.4 ± 1.2 *	5.2 ± 0.9 *	6.6 ± 1.8 *
Infected C57BL6-Kit$^{+/+}$	10	15.1 ± 4.9 *§	4,155 ± 5,200 *	6.3 ± 0.8 *	5.5 ± 1.1 *	7.5 ± 2.3 *
Infected Kit$^{W-sh/W-sh}$	8	23.2 ± 4.6 *	3,427 ± 1,954 *	6.4 ± 0.4 *	5.8 ± 0.9 *	6.3 ± 2.1 *
Infected Kit$^{W-sh/W-sh}$, reconstituted with MC	8	19.1 ± 5.4 *	4,586 ± 3,469 *	6.4 ± 0.7 *	5.7 ± 1.2 *	6.9 ± 3.0 *

Legend: mast cell (MC)-deficient mouse strains = Kit^{W}/Kit^{W-v} and Kit$^{W-sh/W-sh}$. Kit$^{+/+}$ = mast cell (MC) possessing mice. MC reconstitution was done by intracisternal injection of 10^{6} bone-marrow derived MC eight weeks prior to infection. cfu = colony forming units. * P < 0.05, as compared to PBS controls, # P < = 0.05, as compared to infected Kit^{W}/Kit^{W-v} mice, § P < 0.05, as compared to infected Kit$^{W-sh/W-sh}$, using ANOVA and Student-Newman-Keuls posthoc test.

Conclusion: Despite murine bone marrow-derived MC are activated by *S. pneumoniae in vitro*, they do not or only to a minor extent influence the clinical course of PM.