

· 烧伤后炎症反应 ·

G 蛋白不同亚型在严重烫伤小鼠补体活化巨噬细胞分泌功能中的作用

胡远兵 彭代智 黄文华 黎鳌 周新

【摘要】 目的 观察严重烫伤后活化的补体对小鼠腹腔巨噬细胞(PM ϕ)分泌一氧化氮(NO)和肿瘤坏死因子(TNF) α 的影响,探讨信号传递途径中不同 G 蛋白亚型的作用。方法 血浆采集分组:补体血浆组,采用小鼠 18% TBSA III 度烫伤模型;去补体血浆组,先在小鼠腹腔注射眼镜蛇毒素因子(CVF)去补体后再按上述标准烫伤。伤后 6 h 分别收集两组小鼠全血制备血浆,用于培养正常小鼠的 PM ϕ 及经抑制型 G 蛋白(Gi)阻断剂百日咳毒素(PT)预处理的 PM ϕ 、经刺激型 G 蛋白(Gs)激活剂霍乱毒素(CT)预处理的 PM ϕ ,观察各组细胞培养上清液中 NO 及 TNF- α 含量的变化。结果 补体血浆组培养上清液中的 NO 和 TNF- α 含量分别为(80 \pm 12) μ mol/L 和(46 \pm 6)%,明显高于去补体血浆组的(34 \pm 5) μ mol/L 和(26 \pm 5)%($P < 0.01$)。PT 预处理后补体血浆组 PM ϕ 产生的 NO 明显下降[(45 \pm 10) μ mol/L, $P < 0.01$],而 TNF- α 活性[(58 \pm 10)%]增加($P < 0.05$),CT 预处理后补体血浆组 PM ϕ 产生的 NO 增加[(105 \pm 18) μ mol/L, $P < 0.05$],TNF- α 的活性[(27 \pm 6)%]降低($P < 0.01$)。结论 严重烫伤后活化补体引起 PM ϕ 分泌 NO 和 TNF- α 增多这一现象,至少部分是通过 G 蛋白途径实现的。其中对 PM ϕ 生成 NO 的调控主要是通过 Gi 蛋白途径发挥作用,对 PM ϕ 分泌 TNF- α 的调控则以 Gs 蛋白信号通路为主。

【关键词】 烧伤; 补体; 巨噬细胞; GTP 结合蛋白质类; 一氧化氮; 肿瘤坏死因子 α

The role of different subtypes of G protein in the secretory function of macrophages stimulated by activated complement following severe burn injury HU Yuan-bing, PENG Dai-zhi, HUANG Wen-hua, LI Ao, ZHOU Xin. Institute of Burn Research, State Key Laboratory of Trauma, Burns and Combined Injury, Southwest Hospital, The Third Military Medical University, Chongqing 400038, P. R. China
Corresponding author: PENG Dai-zhi, Email: dzpeng@yahoo.com, Tel: 023-68754176

【Abstract】 Objective To investigate the influence of activated complement on the secretory function of peritoneal macrophage (PM ϕ) in the production of nitric oxide(NO) and tumor necrosis factor- α (TNF- α), especially in the role of different G-protein subtypes in this process after burns. Methods The mice inflicted by 18% TBSA full-thickness scald was established and employed as the model. And the mice were divided into A (the complements were preserved and activated) and B (with intraperitoneal injection of CVF to deplete complement before scald) groups. The plasma of the mice in the two groups was collected at 6 postburn hour (PBH) and cultured with PM ϕ from normal mice. The PM ϕ were pretreated with pertussis toxin (PT) and with cholera toxin (CT). The NO and TNF- α levels in the supernatant of normal PM ϕ culture with different pretreatment were measured by Greiss assay. Results The NO and TNF- α contents in group A [(80 \pm 12) μ mol/L, (46 \pm 6)%] were obviously higher than those in group B [(34 \pm 5) μ mol/L, (26 \pm 5)%, $P < 0.01$]. The NO content produced by PM ϕ (45 \pm 10 μ mol/L) in A group decreased ($P < 0.01$), while the TNF- α activity (58 \pm 10)% increased by PT pretreatment ($P < 0.05$). On the contrary, the NO content produced by PM ϕ (105 \pm 18 μ mol/L) in group A increased ($P < 0.01$), while the TNF- α activity (27 \pm 6)% decreased by CT pretreatment ($P < 0.01$). Conclusion These results indicates that the secretory function of normal PM ϕ can be enhanced by complement activation after thermal injury, which might partly be due to the effect of activated complement components through complement receptor coupled G-protein. In the secretory function of complement stimulated M ϕ , Gi protein has a major role in the production of NO, Gs protein is mainly involved in the secretion of TNF- α .

【Key words】 Burns; Complement; Macrophages; GTP binding protein; Nitric oxide; Tumor necrosis factor-alpha

基金项目:国家自然科学基金重大资助项目(39290700-01),全军“九五”医学科研规划重点课题资助项目(96L042)

作者单位:400038 重庆,第三军医大学西南医院全军烧伤研究所,创伤、烧伤与复合伤国家重点实验室(胡远兵现在武警成都医院内一科,610041)

通信(讯)作者:彭代智,Email:dzpeng@yahoo.com,电话:023-68754176

转导途径激活巨噬细胞,其中 G 蛋白途径中的 Gi 和 Gs 很可能都发挥了重要作用。本实验结果表明,补体活化片段通过 Gi 发挥作用,补体活化片段与 Gs 的关系至今鲜见报道。由于信号转导途径和 G 蛋白调节的复杂性,目前要完全弄清楚补体的信号转导途径还有困难,补体活化 PM ϕ 的信号传递亦有待更深入的研究。

参 考 文 献

- 1 胡远兵,彭代智,黄文华,等. 严重烧伤后补体活化的动态变化及对巨噬细胞分泌功能的影响. 中华烧伤杂志,2000,16:231-233.
- 2 Luo G, Peng D, Zheng J, et al. The role of NO in macrophage dysfunction at early stage after burn injury. Burns, 2005, 31: 138-144.
- 3 Schwacha MG, Chaudry IH, Alexander M. Regulation of macrophage IL-10 production postinjury via beta2 integrin signaling and the P38 MAP kinase pathway. Shock, 2003, 20:529-535.
- 4 胡远兵,彭代智,黄文华,等. 严重烧伤后巨噬细胞膜蛋白 GTP 酶活性变化及补体对其影响. 白求恩医学院学报,2003,1:147-149.
- 5 Corcoran ML, Stevenson WGS, Witt DLD, et al. Effect of cholera toxin and pertussis toxin on prostaglandin H synthase-2, prostaglandin E2, and matrix metalloproteinase production by human monocytes. Arch Bio Biophys, 1994, 310: 481-488.
- 6 汪正清,鲜尽红,罗雪,等. 重度烧伤小鼠血清及去补体后血清诱导巨噬细胞凋亡作用的体外实验研究. 免疫学杂志,2004,20:475-477,482.

- 7 彭代智,黄文华. 烧伤后失控性炎症反应的巨噬细胞信号转导机制研究进展. 第三军医大学学报,2004,26:2168-2170.
- 8 Peng DZ, McManus AT, Hu Y, et al. Complement activation stimulate GTPase activity and secretory function of macrophages following burns. J Burn Care Rehabil, 2001,22(2Suppl):126.
- 9 王迎伟,徐静华,汤仁仙,等. 抗胸腺细胞血清性肾炎模型大鼠肾小球中 C5b-9 的沉积及 NO、TNF- α 含量分析. 中国病理生理杂志,2004,20:54-59.
- 10 Gilman AG. G proteins: transducers of receptor-generated signals. Ann Rev Biochem, 1987, 56: 615-649.
- 11 Linder ME, Gilman AG. Purification of recombinant Gi α and Go α proteins from Escherichia coli. Methods Enzymol, 1991, 195: 202-206.
- 12 Norguer L, Dohos G, Konatzki E, et al. Complement fragment C3a stimulates Ca²⁺ influx in neutrophils via a pertussis-toxin-sensitive G-protein. J Biochem, 1993, 217: 289-294.
- 13 Fazal N, Al-Ghoul WM, Schmidt MJ, et al. Lyn-and EPK-mediated vs. Ca²⁺-mediated neutrophil O₂-responses with thermal injury. Am J Physiol Cell Physiol, 2002, 283: 1469-1479.
- 14 Katakami Y, Nkao Y, Koizumi N, et al. Regulation on TNF production by mouse peritoneal macrophages: the role of cellular cyclin AMP. Immunology, 1988, 64: 719-724.
- 15 Murthy KS, Makhlof GM. Vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide-dependent activation of membrane-bound NO synthase in smooth muscle mediated by pertussis toxin-sensitive Gi1-2. J Biol Chem, 1994, 269: 15977-15980.

(收稿日期:2004-11-12)

(本文编辑:王旭)

· 技术与方法 ·

微粒皮制作实用方法介绍

戴海华 海恒林 华云飞 边琳芬 吴胜刚 李强 张志扬 李克荣

改装刀具:去除多用轧皮机刀具(SZS-79-4C型,上海手术器械厂)的刀间隔层,将刀片并拢间隔缩短为1mm,制成轧制微粒皮的刀具。操作方法如同加工邮票状皮肤,制备的微粒皮规格为1mm×1mm。利用新鲜猪皮制备微粒皮时,注意大张猪皮的厚度应控制在1mm。

移植方法:处理烧伤创面至待植皮状态,充分止血。参照文献[1]轻提绸布,将漂浮在等渗盐水中的微粒皮转移至用以覆盖创面的新鲜猪皮真皮面上,再将带微粒皮的猪皮真皮面贴敷于创面。间断缝合固定,用厚层无菌敷料适当加压包扎,避免过早拆开敷料错动尚未黏附牢固的猪皮。通常术后5d首次检视创面,若无明显的局部或全身感染,亦可延至7d后。猪皮下如有积血积液,可作局部切开引流并继续包扎。术后10d左右,微粒皮已开始扩展为可辨认的皮片,其上覆盖的猪皮表现为局部干结。术后3周,微粒皮继续扩展、增厚并融合成片,覆盖自体皮的猪皮逐渐脱落,其下仅为创面的猪皮可持续成活4~6周。

临床应用及结果:笔者对38例大面积深度烧伤患者的

创面按照上述方法进行微粒皮移植手术,供、植皮面积比平均为1.0:(9.0±2.7),微粒皮移植术区占患者总体表面积的(45±18)%,占烧伤总面积的74%。微粒皮成活面积占总植皮面积的(90±5)%,约95%创面封闭时的平均手术次数为6.5次[伤后(42±5)d]。脓毒症和内脏并发症发生率为28.5%,患者生存率为94.7%。

讨论 将原有轧皮机刀具简单改装后制备微粒皮,可明显降低劳动强度、提高工作效率,微粒皮均匀、漂浮同向性好,方便按植皮面积调整供皮量。

以新鲜猪皮作微粒皮的覆盖物,具有取材方便、费用低、制作容易、随时可用等优点^[2]。但应注意新鲜猪皮必须先消毒后加工,以增强与自体微粒皮和创面之间的黏附效果。

参 考 文 献

- 1 张明良,曹大鑫,常致德,等. 微粒体皮肤移植在大面积烧伤病人中应用. 中华整形烧伤外科杂志,1987,3:100-102.
- 2 李克荣,郑冠吾,戴海华. 新鲜猪皮覆盖自体微粒皮修复大面积Ⅲ度烧伤. 中华整形烧伤外科杂志. 1995,11:170.

(收稿日期:2005-04-11)

(本文编辑:王旭)

作者单位:313000 湖州,解放军第九十八医院烧伤整形科