Observation on and correlative analysis of changes in plasma levels of D-dimer, thrombomodulin and free protein S in severely burnt patients*

Tan Wenfeng, Yang Zongcheng, Wang Jiahan, Peng Yizhi, Peng Daizhi, Luo Qizhi, Yu Bin, Li Ao (Research Institute of Burns, Southwest Hospital, Third Military Medical University, Chongqing, 400038)

Abstract Objective: To explore the effects of vascular endothelial cell damage on postburn DIC development in severely burnt patients. Methods: The plasma antigen levels of thrombomodulin, free protein S and D-dimer were determine on the 1st, 3rd, 5th and 7th day following injury in 10 patients (18~45 years old) suffered from 40~50% TBSA full-thickness skin burns. Ten healthy male volunteers were selected as the controls. Results: The plasma levels of thrombomodulin and D-dimer were persistently increased on the 1st, 3rd, 5th and 7th day postburn and there was significantly positive correlation between these two. The plasma level of free protein S was decreased on the 1st day, then gradually increased and still lower than that in the controls on the 7th day postburn. It was negatively correlated to that of D-dimer. Conclusion: Endothelial cell damage and diminution of the effects of protein C anticoagulant pathway are important reasons for DIC development following burns.

Key words burns; thrombomudulin; D-dimer; protein S; endothelial cell

* This project was supported by the National Natural Science Foundation of China, No. 39290700

低速离心法去除细胞培养中霉菌污染 个案与短篇

Eliminating the mycete pollution in the cell culture by low-speed centrifugation

杨 卫兵* 刁庆春 叶庆佾 (第三军医大学附属西南医院皮肤科) 重庆, 400038

细胞培养过程出现霉菌污染是一常见而又非常棘手的问题。对于污染的培养细胞,除了将它们注入动物体内除菌外[1],尚未见其他有效的清除霉菌污染的报道。我们成功地尝试了一种简易的清除霉菌污染的方法。

1 材料与方法

1.1 细胞培养中霉菌污染的确定

取疑有污染的杂交瘤细胞置于 RPMI-1640培养液进行传代培养,于培养第 2~3天后于倒置显微镜下观察细胞形态,离心后进行 PAS染色霉菌镜检及霉菌培养(沙氏琼脂培养基,于温室和 37℃下常规培养 1周,以下霉菌培养条件同此)。

1.2 动物体内除菌法去除细胞培养中的霉菌污染

将霉菌污染的杂交瘤细胞注入 Balb/c小鼠腹腔中,待小鼠腹部膨隆后收集腹水,离心(1 000 r/min)后,弃上清液,得细胞沉淀,RPMI-1640培养液稀释,显微镜下观察细胞成份,同时行 HE染色、革兰氏染色、PAS染色后霉菌镜检及霉菌培养。

1.3 两性霉素 B对细胞培养中霉菌污染的作用

于培养液中分别加入浓度为 10μg/ml、5μg/ml、3μg/ml、1μg/ml的两性霉素 B,每日观察细胞形态。

1.4 低速离心法去除细胞培养中的霉菌污染

将以上从 Balb/c小鼠腹水中得到的存在霉菌污染的杂交瘤细胞置于无血清 PMRI-1640培养液中,进行低速离心(500~800 r/min),每次离心后弃上清液,用消毒棉签吸净离心管中残存液体,并用棉签轻轻吸掉细胞沉淀的表层细胞。如此反复 6~9次,每次离心前后均于显微镜下观察细胞。将以上反复低速离心后的细胞于 RPMI-1640培养液中进行传代培养,同时行霉菌培养,每日观察细胞形态。

2 结果

2.1 细胞培养过程中霉菌污染的确定

有污染的杂交瘤细胞进行培养,第 2天细胞贴壁差,镜下见大量漂浮细胞,细胞膜不光滑,培养液尚清亮;培养至第 3天,培养液变浑浊,镜下可见许多微小生物的布郎运动,细胞死亡;PAS染色镜检证明有菌存在,霉菌培养证实为白色念珠菌。

(下转 553页)

* 杨卫兵,男,30岁,主治医师,硕士,现在解放军 181医院皮肤科 桂林, 541002

?1994-2016 China Academic Journal Electronic Publishing House. All rights reserved. http://www.cnki.net

clinical outcome of HBV infection. **Methods**: 3 groups of HBV infected cases including 3 cases of acute HBV infection with complete recovery, 3 cases of chronic HBV carrier and 3 cases of chronic severe hepatitis B were studied. The preS genes were amplified from serum samples with half nested PCR and the products were cloned into M13 vectors. 10 clones were randomly selected from each case and sequenced with standard procedures. A total of 90 clones were sequenced and analyzed with DNA homology comparison. **Results**: There were different characteristics in HBV infected cases with different outcomes: ① In case of acute infection, the great indentity of DNA sequences among the clones were accompanied with stable function domains without changes of epitopes for T and B cells. ② In the chronic HBV carriers, the preS function domains showed high changes with epitope dreifts of T and B cells. ③ In case of chronic severe HBV infection, special changes existed in the function domains of B cells, which may be the cause of abnormally high immune response and severe liver damages. **Conclusion**: Our findings confirm the fact that there are relations of the changes of the pre S1/S2 function domains with the clinical outcome of HBV infections.

Key words　HBV; Pre S1/S2; function domain; clinical outcome

(上接 549页)

2.2　动物体内除菌法对细胞培养中霉菌污染的作用

将污染的杂交瘤细胞注入 Balb/c小鼠腹腔中,14 d后每只小鼠可得黄色浑浊腹水 3~ 5 ml,离心(1000转/分)后得到乳白色细胞沉淀,HE染色、革兰氏染色、PAS染色霉菌镜检证明杂交瘤细胞中混杂有较多的淋巴细胞、红细胞及霉菌孢子,霉菌培养证实为白色念珠菌;如此反复处理 4次后霉菌污染仍然存在,杂交瘤细胞不能进行传代培养(细胞死亡)。

2.3　两性霉素 B对细胞培养中霉菌污染的作用

将以上从小鼠腹水中得到的细胞置于含有不同浓度的两性霉素 B的细胞培养液中进行培养,5μg/ml和 10μg/ml的两性霉素 B导致细胞死亡,镜下可见大量细胞碎片;而 3μg/ml和 1μg/ml的两性霉素不能抑制霉素生长,于培养第 3天培养液变浑浊,镜下可见细胞死亡及大量布郎运动的微小生物。

2.4　低速离心法去除细胞培养中的霉菌污染

将以上从 Balb/c鼠腹水中得到的杂交瘤细胞进行反复(6~ 9次)低速离心(500~ 800转/min),多次离心及棉签反复处理后,显微镜下可见杂交瘤细胞中混杂的其他细胞成份明显逐步减少,于第 3~ 5次离心后于镜下消失,再离心及棉签处理 3次,最后将细胞置于 RPMI-1640细胞培养液中进行培养,镜下见细胞生长良好,于第 3天呈明显克隆化生长;将以上低速离心处理后的细胞进行传代培养后再行霉菌培养,传代细胞生长良好,霉菌培养未见霉菌生长,霉菌污染完全清除。

3　讨论

细胞培养过程一旦出现霉菌污染,人们往往显得束手无策。镜下观察,杂交瘤细胞与霉菌孢子相比较,二者体积相差悬殊,我们由此得到启示:利用二者沉降系数间的差异,应该能够将其分离。为此我们使用了低速离心法成功地去除了细胞培养中的霉菌污染。我们的体会是:① 离心速度不宜过快,以 500~ 800 r/min为宜,低速离心情况下,体积较大的杂交瘤细胞最先沉淀于离心管的底部,霉菌及红细胞、淋巴细胞沉淀于其表面;② 应予以多次离心,并于每次离心后用棉签吸净离心管中残存的液体,同时吸掉细胞沉淀的表层细胞。于每次离心和棉签处理后观察处理的效果,至镜下见不到霉菌和其它细胞成份,再离心和棉签处理 2~ 3次,然后进行细胞培养;③ 每次离心处理后都可去除部分霉菌污染,但同时也使数量减少,本方法所需细胞量大,离心前应将细胞注入动物体内扩增;④ 两性霉素 B对细胞培养中霉菌污染的作用有限,浓度过高可致细胞死亡,浓度过低时则不能抑制霉菌生长;⑤ 细胞培养中出现霉菌污染时,将细胞注入动物体内借助动物自身的免疫力可消除污染,但该方法所需时间长、所需费用高,如果在较短时间内不能清除杂交瘤细胞中的霉菌污染,将可能影响杂交瘤细胞的生物学功能[1,2];我们使用动物体内除菌法 4次,耗时近 2个月尚能除细胞培养中的霉菌污染,培养的细胞不能生长;⑥ 低速离心法、动物体内除菌法、两性霉素 B除菌法去除细胞培养中的霉菌污染,三者相比较,前者具有明显的优越性:所需实验条件简单,去除霉菌污染彻底、耗时短,能将霉菌污染所造成的对杂交瘤细胞的不良影响降至最低程度。

关键词　细胞培养;低速离心;霉菌污染

中图法分类号　R329-33

参考文献

1 司徒镇强,吴军正 主编. 细胞培养. 北京: 世界图书出版公司, 1996. 85~ 90

2 杨景山. 医学细胞化学与细胞生物技术. 北京: 北京医科大学出版社, 1990. 455~ 457

(收稿: 1998-11-27;修回: 1999-03-31)

(编辑　王　红)

?1994-2016 China Academic Journal Electronic Publishing House. All rights reserved.　http://www.cnki.net