

谷物粉尘致豚鼠哮喘模型支气管肺泡灌洗液 细胞学和肺组织病理学观察

沈阳军区总医院 (110015) 刘相德 陈 萍 康 林 魏路清 王淑云
林小平 董 涓 宋福林 王沙西

提 要 本文对比观察了用谷尘、青霉菌提取液和卵蛋白致敏并激发后阳性反应豚鼠支气管肺泡灌洗液(BALF)内细胞学和肺组织病理学变化。结果发现激发试验阳性的谷尘组、青霉菌组和卵蛋白组哮喘模型BALF内嗜酸细胞数分别为 $36.04 \pm 11.22\%$, $32.21 \pm 8.24\%$ 和 $30.50 \pm 10.20\%$,均非常显著地高于反应阴性组($12.50 \pm 7.29\%$)和对照组($9.75 \pm 4.03\%$), $P < 0.01$ 。光镜下见反应阳性动物支气管、细支气管痉挛以及壁内和腔内大量炎细胞浸润和粘液栓塞等。电镜下可见以嗜酸细胞为主的炎性细胞及其胞浆内大量特异性颗粒及脱颗粒后形成的空泡等。提示嗜酸细胞在谷物粉尘所致豚鼠哮喘的发病过程中起着重要的作用。

关键词 谷物粉尘 青霉菌抗原 哮喘

支气管哮喘的发病不仅与过敏反应有关,

而且还与炎性反应有关。为了探讨谷物粉尘所致职业性哮喘的发病机理,本文对比观察了谷尘、青霉菌和卵蛋白所致哮喘动物模型支气管肺泡灌洗液(BALF)内细胞学及其肺组织病理学变化。

1 材料与方法

1.1 动物致敏和激发 将44只白色短毛豚鼠(雌雄兼用,体重250~350g)随机分组并按下表所示的时间和给药途径致敏动物。致敏后第27天开始各组动物以相应抗原雾化吸入激发。

各组豚鼠致敏途径和时间

组别	动物数	致 敏 原	途 径	时 间
谷尘组	20	50%谷尘提取液和4.4% Al(OH) ₃ 各6ml [▲]	0.45ml i. p. ^{▲▲} 0.15ml × 5s. c. ^{▲▲▲}	第1和第8天
青霉菌组	8	5%青霉菌提取液和4.4% Al(OH) ₃ 各6ml	同 上	同 上
卵蛋白组	8	10%卵蛋白液和4.4% Al(OH) ₃ 各6ml	同 上	同 上
生理盐水组	8	0.9% NaCl和4.4% Al(OH) ₃ 各6ml	同 上	同 上

▲氢氧化铝凝胶(下同) ▲▲腹腔内注射 ▲▲▲颈背和四肢腋下共5点皮下注射

1.2 动物肺功能测定 动物致敏后第27天用戊巴比妥钠(30mg/kg)麻醉后仰卧固定于动物体积描记箱内。在胸骨右缘外侧1~2cm第4肋间隙处剪毛、切皮后,将一端接水检压计和差压传感器(P₁)的16号注射针头(尖部磨钝并开两个侧孔)经肋间隙用力插入并置于胸腔内,检压计水面随呼吸运动而上下波动即确认针头在胸腔内。将豚鼠头部罩上玻璃面罩后接改良式Fleisch呼吸流速器,并接差压传感器P₁的另一端和压力传感器P₂,然后分别接X-Y函数记录仪(四川省重庆仪表厂,LE3—200型)的X轴和Y轴。颈部固定使动物的身体置于封闭的体描箱内。体描箱侧壁上小孔接

压力传感器P₂,并连至Y₂轴。Y₁轴记录流速曲线(F),Y₂轴描记体描箱内随呼吸运动而变化的气体容量,即潮气量(V_T)。X轴记录鼻腔与胸腔内压差(ΔP)。流速用玻璃转子流量计和改良式Fleisch呼吸流速器定标。用“U”型水检压计行压力定标。潮气量定标是用5ml注射器向置于体描箱内的胶皮袋中以一定速度注入定量空气的方法进行。

Y₁轴和X轴同时描记即可记录流速-压力曲线环,它是以产生最大气流速所需最大胸内压力计算肺阻力,即 $R_L = \Delta P / \Delta F$ 。Y₂轴和X轴同时描记容量-压力曲线环,用以计算动态肺顺应性(C_{dyn}),它是动物自主呼吸时,于吸

气开始至吸气终了气流等于零时所测定的潮气量与压差之比,即 $C_{dyn} = \Delta V / \Delta P$ 。

1.3 激发试验阳性判定标准 激发后动物出现呼吸急促、咳嗽,眼球、口唇和双耳紫绀。肺功能检测肺总阻力(R_T)增加 $\geq 100\%$ 或/和动态肺顺应性(C_{dyn})下降 $\geq 40\%$ 者为哮喘阳性反应。

1.4 BALF细胞学检查 将动物激发并监测肺功能3小时后,经颈总动脉放血处死。将头钝的长针插入至叶支气管以下,双侧做灌洗,每侧灌洗3次,每次注入约5ml生理盐水,回收约10ml BALF,立即送检。经双层纱布过滤,取滤液0.5ml加Hank's液至3ml(6倍稀释)加样0.5ml,甩片(1200rpm, 8分钟)后固定,HE染色,油镜下计细胞总数和分类。

用致敏红细胞花环试剂法(武汉生物制品研究所)做T淋巴细胞亚群分类。

1.5 组织病理学检查 用反应阳性和阴性组及对照组动物各两只,取双肺上下叶各约 $2 \times 1 \times 1\text{mm}^3$ 大小的组织块,戊二醛固定做超薄切片,并在透射电镜下观察。余下肺组织用甲醛液固定送做普通病理切片光镜下观察。

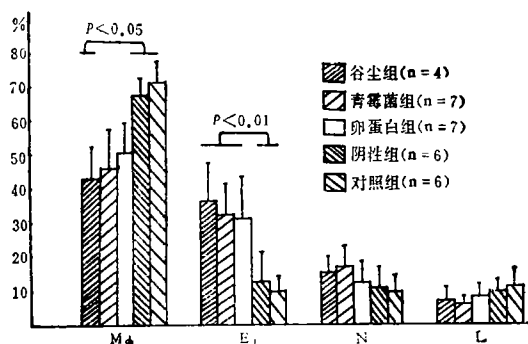
1.6 统计学处理 以Epson PC/XT电子计算机用“医学统计软盘”进行 t 检验。

2 结果

2.1 激发试验阳性率 谷尘组为20%(4/20),青霉菌组为87.5%(7/8),卵蛋白组为87.5%(7/8)。

2.2 BALF内白细胞总数、分类以及T细胞亚群分类 如图所示谷尘组、青霉菌组和卵蛋白组中激发试验阳性动物BALF内嗜酸细胞百分数分别为 $36.04 \pm 11.22\%$ 、 $32.21 \pm 8.24\%$ 和 $30.50 \pm 10.20\%$,均比阴性组($12.50 \pm 7.29\%$)和对照组($9.75 \pm 4.03\%$)非常显著地增高($P < 0.01$)。谷尘和青霉菌激发反应阳性组BALF内巨噬细胞百分数分别为 $42.95 \pm 9.25\%$ 和 $45.50 \pm 10.15\%$,均显著低于对照组的 $70.75 \pm 6.98\%$ ($P < 0.05$)。各组BALF内淋巴细胞数最少,而且T淋巴细胞亚群数在观察组和对照组

BALF内细胞百分数



各组间 BALF 内巨噬细胞(M_ϕ)、嗜酸细胞(E_0)、嗜中性粒细胞(N)和淋巴细胞(L)百分数比较

之间无显著性差异。

2.3 肺组织病理学检查 激发阳性的动物双肺大体标本呈明显肿胀,颜色灰白。光镜下见谷尘、青霉菌和卵蛋白激发反应阳性动物的支气管、细支气管痉挛,管壁和管腔内大量炎细胞渗出、粘液栓塞等,严重者肺泡腔实变。电镜下见肺泡上皮细胞完整,可见嗜酸细胞为主的炎性细胞浸润以及嗜酸细胞胞浆内大量特异性颗粒和脱颗粒后形成的空泡,偶见中性粒细胞。

3 讨论

谷物粉尘是非常复杂的混合物,含有各种谷物壳颗粒、谷物穗和纤毛、淀粉颗粒、霉菌孢子、昆虫碎片(如螨类)、花粉、鼠类和鸟类毛发及其排泄物以及各种化学农药和矿物质颗粒等^[1]。其中谷物壳、霉菌和螨类等具有较强的致敏作用和致喘作用^[2]。本实验表明谷尘和青霉菌同卵蛋白一样均可致豚鼠哮喘发作,其致喘阳性率分别为20%、87.5%和87.5%。

支气管哮喘不仅是过敏性疾病,而且还与呼吸道局部炎症反应有关,其中以嗜酸性细胞的作用为著。过去认为嗜酸细胞的主要作用是杀伤入侵宿主体内的寄生虫,现在发现嗜酸细胞胞浆内所含特异性颗粒,如主碱基蛋白(MBP)、嗜酸细胞阳离子蛋白(ECP)、嗜酸细胞过氧化酶(EPO)等不仅能杀伤外来的寄生

虫,而且还对机体的组织细胞具有较强的损伤作用。嗜酸细胞激活后脱颗粒时将上述物质释放至细胞外,引起气道上皮损伤、激活嗜碱性细胞和肥大细胞释放过敏性介质、刺激神经末梢和血管通透性增加等,从而使支气管、细支气管粘膜肿胀、粘液分泌过多和支气管痉挛等病理改变。此外,脂氧化酶和环氧化酶被激活后使嗜酸细胞膜产生白三烯类(LTC₄、LTD₄)和血栓素(TAX)等花生四烯酸的代谢产物从而加重支气管痉挛和炎症反应。本文谷尘、青霉菌和卵蛋白组动物BALF内嗜酸细胞比例非常显著地增高;光镜下支气管痉挛和管腔及壁内大量炎性细胞渗出和浸润;电镜下见较多的嗜酸细胞及其胞浆内大量特异性颗粒和一些脱颗粒后形成的空泡等改变均提示嗜酸细胞在局部浸润并脱出的颗粒可能在谷尘和青霉菌等所致哮喘发病过程中起着重要作用。

淋巴细胞(主要是CD₄⁺ T淋巴细胞)被活

化后通过释放白细胞介素3(IL-3)、白细胞介素5(IL-5)和中性粒细胞和巨噬细胞集落刺激因子(GM-CSF)等细胞因子而在哮喘的发病过程中起着一定的作用。IL-5在嗜酸细胞的后期成熟和分化阶段是一个不可缺少的因子^[3],而且其主要来源是活化性CD₄⁺ T淋巴细胞。本文观察组BALF内T_H(辅助型T细胞)百分数较对照组略高,但无统计学意义,因此,淋巴细胞的作用尚需进一步研究。

4 参考文献

- 1 Brooks SM, et al. Reactive airways dysfunction syndrome(RADS). A newly defined occupational disease (abstract). Am Rev Respir Dis 1981; 123(Suppl A), 133
- 2 Chan—Yeung M. Occupational Asthma. Chest 1990; 98(5 Suppl):1485~1615
- 3 大嶋勇成 他.好酸球の分化とサイトカイン.臨床免疫 1992;24(4),508~515

脱离粉尘作业工程兵矽肺1例报告

河北省廊坊市卫生防疫站(102800) 刘俊毅 郑双山 周保芹

患者男,57岁,干部,X线胸片号0008。主因咳嗽、咳痰、胸闷,曾在某医院诊治。1991年复查怀疑尘肺,遂于1992年11月来我站。

患者于1956年3月至1961年7月一直担任北京某空军连队工程兵风钻工,此后一直脱离粉尘作业至今。不吸烟,既往健康。

体格检查:血压18/12kPa,营养中等,无杵状指、紫绀,心脏未见异常,两肺呼吸音粗糙,肝脾未触及。

肺功能测定:VC 56.8%,FEV₁ 46.2%,MVV 58.2%,属中度混合性通气障碍。

实验室检查:血尿常规、血沉均在正常范围,心电图正常。

X线胸片所见:两侧肺门轻度扩大,密度增高,结构紊乱;两侧肺纹理增多,增粗,部分扭曲变形;两中下肺区有类圆形小阴影,间有不规则小阴影(p/密集度Ⅰ级;主动脉弓突出。胸膜未见改变,心正常。

综上所述,本例诊断为Ⅰ期矽肺(迟发性)。

讨论 本文报道的迟发矽肺,实际接尘工龄只有5.33年,离尘后至确诊矽肺时间长达31年。说明离尘后迟发性尘肺的发生年限有的要经过很长时期。因此,对这类病例应长期动态追踪观察,不仅几年、十几年,有的要三四十之久。

本病例在50年代中末期曾作为国防工程兵从事风钻工,当时湿式凿岩还不普遍,以干式为主,粉尘浓度很高,劳动条件差,实际接尘5年多,这表明与当时情况是相符的。

接尘作业工人定期健康检查,我国已有规定,而对离尘后从事非粉尘工作,包括转业退伍人员,却被忽视,甚至遗忘,建议今后也应规定这部分人员作为定期健康检查的对象。对于这类病人诊断中要特别注意询问职业史,搜集和调查流行病学资料,加上良好的X线胸片的改变特点,一般诊断不难。另外,迟发性尘肺的存在,进一步证实尘肺病的危害性,为今后如何加强做好尘肺病防治和管理工作,提供了重要内容。

Abstracts of Original Articles

A Study on Effect of Noise on Neurobehavior of Female Textile Workers

Fan Guangqin, et al

Effect of noise neurobehavior of female textile workers was reported. 96 female workers in a textile mill (noise level 86.7 dB~95.8dB) were chosen as the exposure group, 40 healthy subjects worked in the same textile mill (noise level 68.2dB) were used as control group. The proportion of age and educational level between the exposed and control groups were closely similar. According to the exposure level, the exposure group was subdivided into two, i.e. high level and intermediate level groups. All subjects were strictly tested with WHO Neurobehavioral Core Test Battery (WHO NCTB).

Results showed, The neurobehavioral functions of exposed group such as mood state, simple reaction time, digit span, digit symbol, Benton visual retention and pursuit aiming I had significant changed, as compared with the control group. There was a relationship between exposure level and alteration of some behavior.

Key words, noise, the performance in neurobehavior, female workers

Epidemiological Survey for Occupational Chronic Musculo-Skeletal Injury of Mechanical Industrial Workers

Sun Xu, et al

The hundred and fifty eight mechanical industrial workers were examined for musculo-skeletal system in this epidemiological survey. It was found that 63.7% and 49.16% of these workers were occupational chronic musculo-skeletal injury and low back pain respectively. Both differed significantly from those of

the control group. There were positive correlation between chronic musculo-skeletal injury prevalence and length of employment.

Key words, mechanical industry, occupational chronic musculo-skeletal injury, occupational low back pain

Occupational Trinitrotoluene — Exposed Effects on Semen—Quality in the Workers

Liu Haixi, et al

The result showed that the concentrations of Trinitrotoluene (TNT) in air of the workshop were higher than that of Maximum Allowable Concentrations (MAC) and the skin of the exposed workers were seriously contaminated by TNT.

The results of measuring the contents of semen zinc, the activities of semen Lactate Dehydrogenase (LDH), the concentrations of serum testosterone (T) and semen qualities of 49 TNT-exposed workers and 50 control workers are listed as follows, the contents of semen LDH and zinc and the concentrations of serum T of TNT-exposed group are significantly lower than that of control group, while the abnormal rates of semen qualities of the exposed workers such as the liquefaction time, shape and survival of semen were higher than that of control workers.

Key words, semen zinc, semen qualities, testosterone, trinitrotoluene, LDH

Observation on the BALF Cytologic and Pulmonary Histopathologic Characteristics in Grain Dust Induced Guinea pig Asthma Models

Liu Xiangde, et al

In this report, the cytologic characteris-

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Number 5

A study on effect of noise on neurobehavior of female textile workers

Fan Guangqin, et al (257)

Epidemiological survey for occupational chronic musculo-skeletal injury of mechanical industrial workers

Sun Xu, et al (260)

Occupational Trinitrotoluene-exposed effects on semen quality in the workers

Liu Haixi, et al (263)

Observation on the BALF cytologic and pulmonary histopathologic characteristics in grain dust induced guinea pig asthma models

Liu Xiangde, et al (266)

tics of bronchoalveolar lavage fluid (BALF) as well as pulmonary histopathologic changes in guinea pigs after being sensitized and challenged with extracts of grain dust, Penicillium SP (extracted from raw grain dust) and ovalbumin, respectively, were reported. It was found that the eosinophil percentage in the BALF of animals showing asthmatic reaction to grain dust or Penicillium SP extracts was $38.00 \pm 11.22\%$, and very significantly higher than that of animals showing negative reaction to the challenges ($12.50 \pm 1.29\%$) and control group ($9.75 \pm 4.01\%$), respectively ($P < 0.01$). Under light microscopy, changes such

as bronchospasm, large quantity of inflammatory cells invading into the bronchial walls or lumen and mucus blocks were observed in the animals with asthmatic reaction. Furthermore, under electronic microscopy, the predominant eosinophils with large amount of characteristic granules or degranulation were found in the pulmonary tissues of asthma models. The findings suggested that eosinophil accumulation followed by airway inflammation may play an important role in the pathogenesis of grain dust induced asthma.

Key words: grain dust, asthma, BALF