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厦门大学

硕　士　学　位　论　文

呼吸道合胞病毒感染动物模型的建立及应用

Establishment and Application of Respiratory Syncytial Virus Animal Model

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摘要

人呼吸道合胞病毒（Human Respiratory SyncytiaVirus, hRSV）是全球范围内引起下呼吸道感染主要病原体之一。其感染人群年龄分布广，新生儿与老年人是主要高危人群。据报道，几乎所有小于 2 周岁婴幼儿都有 RSV 感染经历，婴幼儿发病住院率高达 2.5-4%。RSV 引起大于 65 周岁的老年人感染率为 3-4%，病死率约为 2%。早产儿、先天性心脏病、先天性支气管及肺发育不良、免疫缺陷的婴幼儿 RSV 病死率率高达 5%，造成严重的医疗负担。

RSV 无安全、有效疫苗上市，唯一获准上市的预防性 RSV 药物为中和性单抗 palivizumab (Synagis®)，特异性识别 RSV 融合蛋白。Synagis 在动物体内预防性试验的成功提示 RSV 感染动物模型的建立有助于疫苗、抗体药物的保护性研究。RSV 感染动物模型种类繁多，如小鼠、棉鼠、雪貂、猩猩等，每种动物都有其感染特征。其中，小鼠模型由于易获得近交系及研究用检测试剂等，最常用于 RSV 感染研究，又以 BALB/c 小鼠应用最广；裸鼠适应性免疫系统缺陷，感染后鼻、肺内病毒清除延迟，因此可研究先天免疫介导 RSV 引起的气道炎症应答，模拟免疫缺陷人群 RSV 感染。

本研究建立不同周龄 BALB/c 小鼠及裸鼠模型，模拟不同易感人群 RSV 感染后症状，运用该模型对 RSV 特异性抗体进行体内预防性评估，为 RSV 药物的开发奠定基础。首先，本论文分别选取 10 周龄、30 周龄、60 周龄 BALB/c 小鼠，分别感染 100 μL 的 10^7 PFU/mL、10^8 PFU/mL 滴度的 RSV 后比较其临床症状及病理变化。结果显示，感染 10^8 PFU/mL 滴度的 RSV 感染后不同周龄小鼠都有明显的体重下降；鼻、肺检测到较高滴度病毒；免疫组织化学检测抗原定位于肺泡、支气管周围的炎症细胞胞浆；引起明显的炎症细胞浸润、肺组织病理损伤；随着小鼠周龄的增大，RSV 感染后引起的临床症状及病理变化越严重。进一步感染 10 周龄裸鼠，旨在模拟免疫缺陷 RSV 感染症状，结果发现与 BALB/c 小鼠相比，RSV 并不能使裸鼠体重下降，但裸鼠鼻、肺内病毒滴度高、持续时间久；而裸鼠肺炎症反应出现较 BALB/c 早，感染后第 3 天即出现明显的炎症反应，第 7 天后炎症逐渐减轻、恢复；抗原定位在肺泡、支气管周围，以第 3 天最为明显，第

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12 天鲜见有阳性铁锈色反应。说明适应性免疫缺陷裸鼠可利用天然免疫独立介导 RSV 感染引起的小鼠气道炎症。利用上述已建立好的不同周龄 BALB/c 小鼠模型，以 1129（Synagis 的鼠源性抗体）作为对照抗体，对实验室筛选的一株特异性针对 RSV-F 蛋白的强中和抗体 5C4 进行小鼠体内预防性评估，结果显示，5C4 与 1129 都能有效减轻 RSV 引起的体重下降；降低鼻、肺内病毒滴度；及肺部炎症反应炎症。而 5C4 在低剂量与 1129 高剂量条件下对小鼠预防性效果一致；上述现象在不同周龄鼠呈现一致，说明 5C4 抗体在不同周龄 BALB/c 小鼠上都有良好的预防性效果，其有效剂量为 1129 抗体的 1/10，因此可作为 RSV 预防性药物潜在开发对象。

综上所述本论文建立不同周龄 BALB/c 小鼠、裸鼠感染动物模型，模拟不同人群 RSV 感染临床症状及病理变化，为不同人群 RSV 感染免疫机制的深入研究及不同年龄段相关抗体和疫苗药物的选择、评估提供依据；同时将 RSV 感染的 BALB/c 模型应用于 RSV 潜力预防性抗体的评估，为新一代 RSV 预防性药物的开发提供有效的临床前评估平台。

关键词：呼吸道合胞病毒；动物模型；肺炎支气管炎；中和抗体预防性评估

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Abstract

Respiratory syncytial virus (RSV) is one of major pathogens for lower respiratory tract infection. It has a large infected age distribution, including almost all the population from the newborn to the elderly. It is reported that almost all children under two-year old have been infected by RSV. Hospitalization of infants due to RSV infections is 2.5-4% each year. Data from a variety of studies suggests that adults over 65 years of age, the overall annual incidence of RSV illness is 3 to 4%, with an estimated a fatality rate of 5%. Also, RSV has a reported fatality rate of 5% among premature births, congenital bronchial, pulmonary dysplasia and immune deficiency individuals, causing a significant medical burden.

Yet, there is no effective RSV vaccines on the market, the only approved anti-RSV drug is a neutralizing antibody palivizumab (Palivizumab) which recognizes the RSV fusion protein. Success of palivizumab supported that the building of RSV animal model would be helpful for the protection trails of RSV vaccine and antibody drugs.

There is a various type of RSV animal model, such as mice, cotton rats, ferrets, chimpanzees and so on, each model has its own charateristics. Among them, mice model was used in study of RSV infection most commonly due to easy access to inbred and detection reagent and BALB/c mice were most widely used. As lacked the adaptive immunity and delayed pulmonary viral clearance, nude mice were always used as model to research the innate immunity reponse after RSV infection and stimulate the immunocompromised population.

Our study aimed to build different week-old BALB/c mice model and nude mice model of RSV infection, to simulate infection symptoms of different population and used these models to evaluate the prophylactic potential of RSV antibodies. First, young (10 weeks), middle aged (30 weeks) and aged (60 weeks) mice were intranasally infected with 10^6 plaque-forming units (PFU) or 10^7 PFU RSVA2 strains,

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lung virus titer, histology and immunohistochemistry was examined, and age sensitivity was analyzed. A high-titer virus infection showed an obvious symptom. Mice trended to be more susceptible to RSV infection as the growth of age. Titers up to $10^{6.5}$ PFU/gram lung can be attained in 60-week-old mice. Older mice experience more weight loss. Lung histology of older mice showed more serious bronchiolitis and increased number of inflammatory cells in alveolar spaced compared with that of younger mice. Further, 10 weeks BALB/c mice and nude mice were intranasally infected with 10^7 PFU RSVA2 strains, simulating infection symptom of immune deficiency population. Comparing with BALB/c mice, nude mice showed no weight loss, while $10^{6.5}$ PFU/gram virus titer was attained in nude mice at day3 and $10^{4.3}$PFU/gram maintained at day20. Nude mice showed early bronchiolitis compared with that of BALB/c mice. RSV antigen located around the alveolar and broncho and detected less at day12.

Using these animal model, we aimed to evaluate prophylaxitic potential of pre-F specific antibody 5C4. It was obersvered that compared with 1129, antibody 5C4 exhibited significant advantage in the terms of prophylactic efficacy, in ech week-old group, suggesting that it is potential to be commercialized and replaces the previous product palivizumab. It showed that 5C4 and 1129 can effectively reduce weight loss, lung and nose virus titer and lung pathology induced by RSV. And 5C4 in the low dose had the similar effect with high dose of 1129. The above phenomenon could show in different ages, indicating that antibody 5C4 have a good preventive effect in different week old BALB / c mice and the effective dose is one tenth of 1129 antibody. Therefore, it can be used as RSV potential prophylaxis.

In this study, we successfully established a different week-old BALB/c mice model and nude mice model, simulating the pathology and symptom of different populations after RSV infection and providing a basis for the further study of the immune mechanism in different populations and the selection and evaluation of different age related vaccines. Also, using these animal model, we can evaluate the prophylaxitic potential of highly efficient neutralizing antibody which will serve as the platform for development of new RSV potential antibody.

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Key words: RSV; animal model; pneumonia and bronchitis; prophylaxitic potential evaluation

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缩略词

英文缩写	英文全称	中文全称
hRSV	human respiratory syncytial virus	人类呼吸道合胞病毒
RSV A2	respiratory syncytial virus strain A2	呼吸道合胞病毒 A2 株
RSV-A	subgroup A of respiratory syncytial virus	A 亚型呼吸道合胞病毒
RSV-B	subgroup B of respiratory syncytial virus	B 亚型呼吸道合胞病毒
FI-RSV	formalin inactivated RSV vaccine	福尔马林灭活 RSV 疫苗
DNA	deoxyribonucleic acid	脱氧核苷酸
RNA	ribonucleic acid	核糖核酸
mRNA	messenger ribonucleic acid	信使核糖核酸
G protein	glycoprotein	糖蛋白
F protein	fusion protein	融合蛋白
M protein	matrix protein	基质蛋白
P protein	phosphoprotein	磷蛋白
SH protein	small hydrophobic protein	小疏水蛋白
L protein	large nucleoprotein	大核蛋白
N protein	nucleoprotein	核蛋白
EM	electron microscopy	电子显微镜
kb	kilo base pair	千碱基对
bp	base pair	碱基对
KD	kilo nucleotide	千道尔顿
ddH_2O	ddH_2O	双蒸水
PFU	plaque forming units	嗜空斑形成单位
IgG	Immunoglobulin G	免疫球蛋白 G
mAb	monoclonal antibody	单克隆抗体
Kan	Kanamycin	卡那霉素
Amp	Ampicillin	氨苄西林

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PBS	phosphate buffered saline	磷酸盐溶液
FBS	fetal bovine serum	胎牛血清
FDA	Food and drug Administration	食品药物管理局
HE	hematoxylin-eosin staining	苏木精-伊红染色
IHC	immunohistochemistry	免疫组织化学反应
IFN-γ	γ-interferon	γ-干扰素
NPA	Nasopharyngeal aspirate	鼻咽抽吸物
rpm	Revolutions per minute	每分钟转速
DMSO	Dimethylsulfoxide	二甲基亚砜

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