

# 抗血小板GP I b $\alpha$ 抗体诱导建立原发性血小板减少症模型的研究

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**【摘要】 目的** 探讨应用抗血小板膜糖蛋白GP I b $\alpha$ 抗体AN51、R300建立原发性免疫性血小板减少症(ITP)动物模型。**方法** 将20只6~8周豚鼠和裸鼠分别分为对照组和3个实验组,每组5只。豚鼠对照组和实验组分别静脉注射0.2  $\mu$ g/g IgG和0.05、0.1、0.2  $\mu$ g/g AN51,裸鼠对照组和实验组分别腹腔注射0.2  $\mu$ g/g IgG和0.05、0.1、0.2  $\mu$ g/g R300。在注射后不同时间点对豚鼠和裸鼠行眼眶后静脉丛采血,应用血细胞分析仪检测血小板水平。**结果** ①静脉注射AN51后5 min,0.05、0.1、0.2  $\mu$ g/g AN51组豚鼠血小板计数分别减低0~5%、50%~60%、70%~80%,0.2  $\mu$ g/g组下降最为明显,与对照组比较差异有统计学意义( $P<0.001$ )。②腹腔注射R300后6 h,0.05、0.1、0.2  $\mu$ g/g R300组裸鼠血小板计数分别下降20%~30%、60%~70%、80%~90%,0.2  $\mu$ g/g组下降最为明显,与对照组比较差异有统计学意义( $P<0.001$ )。每天1次持续注射0.2  $\mu$ g/g R300 1~2周的裸鼠出现典型ITP出血症状(全身大量瘀点、瘀斑,多见于四肢、头部和腹部)。**结论** 0.2  $\mu$ g/g AN51静脉注射、0.2  $\mu$ g/g R300腹腔注射可以分别建立豚鼠、裸鼠ITP模型。

**【关键词】** 血小板减少; 模型,动物; 血小板膜糖蛋白I b

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**A Study on the establishment of immune thrombocytopenia model induced by anti-platelet GP I b $\alpha$  antibodies** Zhou Kangxi, Yan Rong, Chen Mengxing, Liu Jun, Cui Qingya, Hu Renping, Liu Yancai, Zhang Yang, Ruan Changgeng, Dai Kesheng. Jiangsu Institute of Hematology, The First Affiliated Hospital of Soochow University; Key Laboratory of Thrombosis and Hemostasis, Ministry of Health; Collaborative Innovation Center of Hematology, Soochow University, Suzhou 215006, China  
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**【Abstract】 Objective** To establish primary immune thrombocytopenia (ITP) animal model induced by anti-platelet membrane glycoprotein GP I b $\alpha$  antibodies AN51 and R300. **Methods** Twenty guinea pigs (6–8 week) were divided into 4 groups. Five guinea pigs in each group were intravenously injected with different doses of AN51 (0.05, 0.1, 0.2  $\mu$ g/g) and 0.2  $\mu$ g/g IgG as control. The whole blood was collected from inner angular venous plexus. Platelets number was determined by an automated cell counter and Swiss-Jim method. Then, the similar protocol was used to establish ITP nude mice model by intraperitoneal injection of different concentrations of anti-platelet GP I b $\alpha$  antibody R300, respectively. **Results** ①Five minutes after intravenous injection of AN51 at 0.05, 0.1 and 0.2  $\mu$ g/g, the platelet counts of guinea pigs reduced about 0–5%, 50%–60% and 70%–80% compared to the control group, respectively. The difference was statistically significant ( $P<0.01$ ). ②Six hours after intraperitoneal injection of R300 at 0.05, 0.1, 0.2  $\mu$ g/g, the platelet counts of nude mice decreased about 20%–30%, 60%–70% and 80%–90% compared to the control group, respectively. The difference was statistically significant ( $P<0.01$ ). The nude mice, injected 0.2  $\mu$ g/g R300 once a day for 2 weeks, showed typical ITP clinical manifestations including large number of petechiae or ecchymoses on limbs, head and abdomen. **Conclusion** AN51 at 0.2  $\mu$ g/g and R300 at 0.2  $\mu$ g/g could establish stable ITP model in guinea pigs and nude mice respectively.

**【Key words】** Thrombocytopenia; Models, animal; Platelet Membrane Glycoprotein I b

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原发性免疫性血小板减少症(ITP)是一种常见的由于自身免疫引起的出血性疾病<sup>[1-3]</sup>。ITP患者体内抗自身血小板抗体多数为抗GP II b/III a型,GP I b-IV型占32.1%<sup>[4-5]</sup>。然而,抗GP I b-IV型抗体的患者多为难治性ITP,常规药物治疗和脾切除的效果不佳<sup>[6-7]</sup>。ITP的发病机制目前还不是十分清楚,其主要是因为机体的免疫系统异常,产生了抗血小板的自身抗体,从而引起自身血小板计数下降<sup>[8]</sup>。本研究我们使用抗血小板膜糖蛋白GP I b $\alpha$ 抗体诱导建立豚鼠和裸鼠ITP模型,旨在为研究ITP发病机制提供理想的动物模型和技术方法。

## 材料与方法

1. 材料:抗血小板膜糖蛋白GP I b $\alpha$ 抗体AN51由江苏省血液研究所阮长耿院士惠赠。抗血小板膜糖蛋白GP I b $\alpha$ 抗体R300购自德国Emfret公司。豚鼠、裸鼠购自昭衍新药研究有限公司。

2. 洗涤血小板:分别采集豚鼠和裸鼠血液2.0、1.0 ml,以葡萄糖柠檬酸钠7:1抗凝后混匀,200  $\times$ g离心20 min,得到富含血小板血浆(PRP),1 700  $\times$ g离心2 min,弃去上清液,沉淀部分用CGS缓冲液(0.123 mol/L NaCl、0.033 mol/L D-葡萄糖、0.013 mol/L 枸橼酸三钠,pH 6.5)重悬,1 700  $\times$ g离心2 min,弃去上清液,沉淀部分重悬于MTB缓冲液,最后调整血小板悬液的终浓度至 $3 \times 10^8$ /ml。调整Ca<sup>2+</sup>、Mg<sup>2+</sup>终浓度为1 mmol/L,室温静置1 h备用。

3. 流式细胞术检测抗体与血小板结合:取100  $\mu$ l  $3 \times 10^8$ /ml豚鼠或裸鼠血小板悬液,分别加入相对应的终浓度10  $\mu$ g/ml 2.5  $\mu$ l IgG(对照组)、1  $\mu$ l AN51(AN51组)和2  $\mu$ l R300(R300组)轻轻混匀,室温下孵育30 min,然后加入200  $\mu$ l CGS缓冲液,1 700  $\times$ g离心2 min,弃去上清液后分别重悬于50  $\mu$ l含有抗IgG、AN51和R300二抗(10  $\mu$ g/ml)的MTB缓冲液,室温孵育30 min,加入350  $\mu$ l MTB缓冲液上流式细胞仪检测,以CD41-PE和侧向角双参数设定血小板门,FL1 Log荧光通道(488 nm)观测抗体与血小板结合百分率(计数阳性细胞10 000个)。

4. 抗血小板膜糖蛋白GP I b $\alpha$ 抗体诱导建立ITP动物模型:将20只6~8周豚鼠和裸鼠分别分为

对照组和3个实验组,每组5只。豚鼠对照组和实验组分别静脉注射0.2  $\mu$ g/g IgG和0.05、0.1、0.2  $\mu$ g/g AN51,裸鼠对照组和实验组分别腹腔注射0.2  $\mu$ g/g IgG和0.05、0.1、0.2  $\mu$ g/g R300,在注射后不同时间点对豚鼠和裸鼠行眼眶后静脉丛采血,应用血细胞分析仪检测血小板水平,以此确定建立ITP模型所需要抗体的剂量及时间。

5. 统计学处理:采用SPSS 18.0软件进行统计学分析。所有数据以均数 $\pm$ 标准差表示,两组之间的比较采用配对*t*检验, $P < 0.05$ 认为差异有统计学意义。

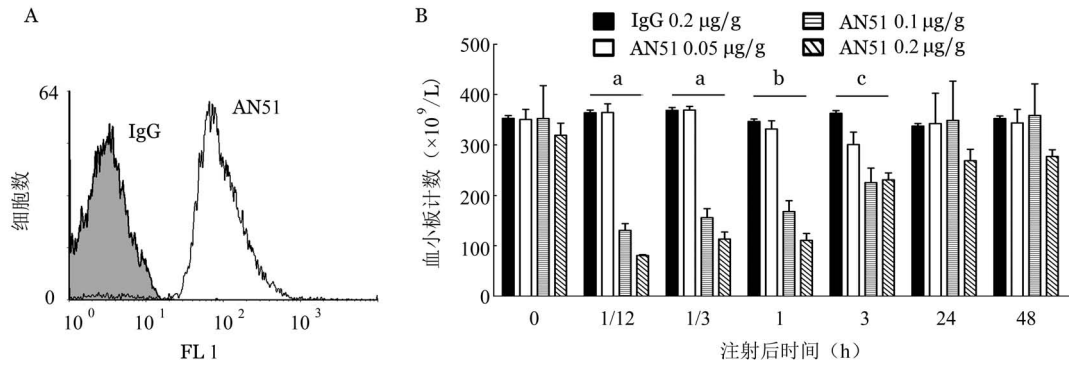
## 结 果

1. AN51诱导建立ITP豚鼠模型:流式细胞术检测显示,AN51能够与豚鼠血小板结合(图1A)。静脉注射0.05、0.1、0.2  $\mu$ g/g AN51后不同时间点行眼眶后静脉丛采血,血细胞分析结果显示AN51能够以剂量依赖性方式诱导豚鼠外周血血小板计数快速下降,注射后5 min,0.05、0.1、0.2  $\mu$ g/g AN51组豚鼠血小板计数分别减低0~5%、50%~60%、70%~80%,0.2  $\mu$ g/g组血小板计数下降最为明显,与对照组(注射0.2  $\mu$ g/g IgG)比较差异有统计学意义( $P < 0.001$ )(图1B)。

2. R300诱导建立ITP裸鼠模型:流式细胞术检测显示,R300能够与裸鼠血小板结合(图2A)。腹腔注射0.05、0.1、0.2  $\mu$ g/g R300后不同时间点行眼眶后静脉丛采血,血细胞分析结果显示R300能够以剂量依赖性方式诱导豚鼠外周血血小板计数快速下降,注射后6 h,0.05、0.1、0.2  $\mu$ g/g R300组裸鼠血小板计数分别下降20%~30%、60%~70%、80%~90%,0.2  $\mu$ g/g组血小板计数下降最为明显,与对照组(注射0.2  $\mu$ g/g IgG)比较,差异有统计学意义( $P < 0.001$ )(图2B)。我们用0.2  $\mu$ g/g R300建立裸鼠ITP模型,每天注射1次,持续注射1~2周后,裸鼠出现典型ITP出血症状(全身大量瘀点、瘀斑,多见于四肢、头部和腹部)(图3)。

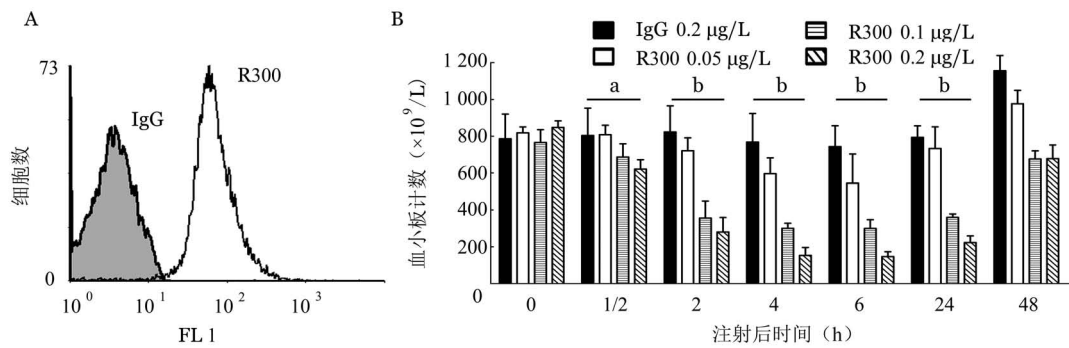
## 讨 论

ITP是一种自身免疫性出血性疾病。其具体发



A:流式细胞术检测 AN51 与豚鼠血小板结合;B:静脉注射 AN51 后不同时间点血小板水平;<sup>a</sup>*P*<0.001, <sup>b</sup>*P*<0.01, <sup>c</sup>*P*<0.05

图1 抗血小板膜糖蛋白 GP I b $\alpha$  抗体 AN51 诱导建立原发性血小板减少症豚鼠模型



A:流式细胞术检测 R300 与裸鼠血小板结合;B:腹腔注射 R300 后不同时间点血小板水平;<sup>a</sup>*P*<0.05, <sup>b</sup>*P*<0.001

图2 抗血小板膜糖蛋白 GP I b $\alpha$  抗体 R300 诱导建立原发性血小板减少症裸鼠模型



图3 腹腔注射抗血小板膜糖蛋白 GP I b $\alpha$  抗体 R300(0.2 µg/g) 诱导建立原发性血小板减少症裸鼠模型

病机制目前尚未阐明,目前主要认为是由于血小板自身抗体致敏的血小板被单核-巨噬细胞系统过度破坏<sup>[9-10]</sup>、自身抗体导致巨核细胞成熟障碍<sup>[11-12]</sup>、细胞毒 T 细胞溶解血小板<sup>[13-14]</sup>和抗原特异性 T 细胞免疫失耐受<sup>[15-18]</sup>等原因引起血小板减少。

本课题组既往研究发现抗血小板 GP I b $\alpha$  氨基端抗体 AN51 能够诱导血小板 GP I b $\alpha$  簇集引起的  $\alpha$  II b $\beta$ 3 依赖的血小板聚集,从而导致血小板被肝脏巨噬细胞所识别,进而在肝脏中被清除<sup>[19]</sup>。本研究

中我们发现 AN51 能够很好地与豚鼠血小板结合, ITP 模型个体之间差异很小,因此很适合用于实验研究。同时,为了建立跟人类 ITP 更加相似的动物模型,我们还使用抗小鼠血小板抗体 R300 成功建立 ITP 裸鼠模型。而且我们还发现,持续注射 R300 抗体的裸鼠出现了明显的临床出血症状。

综上所述,我们应用 0.2 µg/g AN51 和 0.2 µg/g R300 分别成功建立豚鼠、裸鼠 ITP 模型,其中裸鼠模型表现出明显的 ITP 临床症状。

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