·论　著·

乳凝集素抗独特型抗体Ab2β在人轮状病毒腹泻乳鼠模型治疗中的作用

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摘要：目的　探讨乳凝集素抗独特型抗体Ab2β在新生乳鼠感染人轮状病毒（human rotavirus, HRV）的预防与治疗中的作用，并分析其作用机制。**方法**　采用杂交瘤技术制备乳凝集素抗独特型抗体；选取120只7日龄昆明小鼠乳鼠作为研究对象，采用随机数字表法将其分为A、B、C组以及对照组，每组30只，A、B、C三组均采用灌胃病毒方式造模，其中A组造模后不进行任何处理，B组在造模前连续7 d喂服乳凝集素，C组在造模后连续7 d喂服乳凝集素，对照组灌胃不含病毒的细胞培养液。观察各组乳鼠感染病毒后不同时间点的临床表现（腹泻、体重）并采用酶联免疫分析法（enzyme-linked immunosorbent assay, ELISA）检测乳鼠粪便中轮状病毒抗原含量；HRV感染7 d后，采用免疫组化法检测各组乳鼠小肠组织细胞间粘附分子1（intercellular adhesion molecule-1, ICAM-1）的表达水平。**结果**　对照组新生乳鼠各时间点均未非发生腹泻，A、B、C 3组乳鼠在HRV攻击1 d后出现腹泻症状，B、C组腹泻程度在HRV攻击后2~4 d低于A组，差异有统计学意义（$P<0.05$）；B、C组乳鼠粪便中HRV抗原含量在HRV攻击后1~5 d低于A组，差异有统计学意义（$P<0.05$），B、C组各时间点腹泻程度和HRV抗原含量比较，差异均无统计学意义（$P>0.05$）。各组乳鼠感染前、感染后1 d体重比较，差异无统计学意义（$P>0.05$）；B、C组乳鼠在HRV攻击后3、5、7 d体重高于A组，差异有统计学意义（$P<0.05$），B、C组乳鼠在HRV攻击后各时间点体重比较，差异均无统计学意义（$P>0.05$）。A、B、C三组乳鼠在HRV攻击7 d后小肠组织ICAM-1表达细胞数高于对照组，差异有统计学意义（$P<0.05$），B、C组ICAM-1表达细胞数及灰度值低于A组，差异有统计学意义（$P<0.05$）。**结论**　乳凝集素抗独特型抗体Ab2β对新生乳鼠人轮状病毒感染具有很好的预防与治疗作用，可显著改善腹泻症状、降低HRV病毒载量，其具体作用机制可能与抑制小肠组织ICAM-1的表达有关。

关键词：乳凝集素抗独特型抗体Ab2β；人轮状病毒；腹泻；白细胞黏附分子

中图分类号：R373　**文献标识码：**A　**文章编号：**1009-9727(2023)07-736-06

DOI:10.13604/j.cnki.46-1064/r.2023.07.11

Role of lactadherin anti-idiotypic monoclonal antibodies Ab2β in the treatment of human rotavirus-induced diarrhea in a neonatal mouse model

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Abstract: Objective　To investigate the preventive and therapeutic effects of anti-idiotypic monoclonal antibodies (Ab2β) of lactadherin on neonatal mice infected with human rotavirus (HRV), and to analyze the underlying mechanism. **Methods**　Hybridoma technology was used to prepare Ab2β of lactadherin. One hundred and twenty 7-day-old Kunming mice were randomly divided into groups A, B, C and control, each consisting of 30 mice. Groups A, B, and C were all infected with HRV via oral gavage. Group A received no treatment, group B was orally administered lactadherin for 7 days prior to infection, and group C was orally administered lactadherin for 7 days after infection, the control group was orally administered cell culture medium that did not contain the virus. The clinical manifestations (diarrhea, body weight) at different time points after infection of the neonatal mice in each group were observed, and the content of rotavirus antigen in the feces of neonatal mice was detected by enzyme-linked immunosorbent assay (ELISA). After HRV infection for 7 days, immunohistochemical staining was used to examine the expression level of intercellular adhesion molecule-1 (ICAM-1) in mouse small intestinal tissues in each group. **Results**　No diarrhea occurred in the control group at any time point. Groups A, B, and C showed diarrhea symptoms after HRV challenge for 1 day. The degree of diarrhea in groups B and C was lower than that in group A at 2-4 days after HRV challenge, and the difference was statistically significant ($P<0.05$). The HRV antigen content in the feces of the neonatal mice in groups B and C was lower than that in group A at 1-5 days after HRV challenge, and the difference was statistically significant ($P<0.05$). There was no significant difference in the degree of diarrhea and HRV antigen content

基金项目：合肥市卫计委应用医学研究项目（No. hwk2016zc010）

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between groups B and C at each time point (P>0.05). There was no significant difference in the body weight of the neonatal mice in each group before infection and 1 day after infection (P>0.05); the weight of neonatal mice in groups B and C was higher than that in group A at 3, 5 and 7 days after HRV challenge, and the difference was statistically significant (P<0.05), and there was no significant difference in body weight between groups B and C at each time point after HRV challenge (P>0.05). The number of ICAM-1 expressing cells in the small intestine of the three groups A, B, and C was higher than that of the control group after HRV challenge for 7 days, and the difference was statistically significant (P<0.05). The cell number and gray value of ICAM-1 expressing cells in groups B and C were lower than those in group A, and the difference was statistically significant (P<0.05). **Conclusions** Anti-idiotypic monoclonal antibodies (Ab2β) of lactadherin has a good preventive and therapeutic effects on human rotavirus infection in neonatal mice, and can significantly improve diarrhea symptoms and reduce HRV viral load. Its specific mechanism may be related to the inhibition of ICAM-1.

Keywords: Anti-idiotypic monoclonal antibodies (Ab2β) of lactadherin; human rotavirus; diarrhea; leukocyte adhesion molecule

人轮状病毒(human rotavirus, HRV)作为一种双链核糖核酸病毒(呼肠孤病毒科)是引起婴幼儿腹泻的主要病原体之一,HRV经粪口途径传播感染后可导致患儿小肠上皮细胞损伤,并产生肠毒素从而引起胃肠炎,出现以严重腹泻为症状的病毒感染性疾病[1]。相关调查结果显示,仅2013年印度因轮状病毒死亡的患者达47 100人,全球轮状病毒感染的死亡率达到22%,亚非4国死亡率占全球的49%[2],因而轮状病毒感染性腹泻给社会公共卫生安全以及整体经济均带来沉重负担。轮状病毒感染性腹泻的临床治疗难度较大,常规的抗病毒药物在轮状病毒感染性腹泻患者中应用效果并不理想,尤其是机体多系统功能尚未发育完全的婴幼儿,其治疗难度大且预后结局较差。临床研究显示,有效的疫苗接种是预防HRV感染的有效手段,但不同种类的疫苗其预防治疗效果存在较大差异[3]。本组研究基于人母乳中的乳凝集素进行动物实验研究,旨在探讨乳凝集素抗独特型抗体Ab2β对新生乳鼠HRV感染的预防和治疗作用并分析其作用机制。

1 材料与方法

1.1 *实验材料* 实验动物选用7日龄清洁级昆明小鼠乳鼠120只。动物来源于安徽省医学动物中心,购自北京维特莱河实验动物技术有限公司,严格按照国家动物研究使用指南进行。

乳凝集素制备:收集乳汁,在4 ℃ 3 000×g条件下离心处理10 min获取乳清成分,将乳清分装,向乳清中加入naive lysis缓冲液分离获取乳凝集素。用纯化的人乳凝集素与相应的免疫佐剂混合免疫小鼠,得到相应的B淋巴细胞。将得到的B淋巴细胞和小鼠骨髓瘤细胞进行融合杂交,在HAT选择性培养基上筛选杂交瘤阳性克隆细胞,在体内和体外获得单克隆抗体,通过克隆化培养和抗体鉴定,用杂交瘤细胞产生的单克隆抗体免疫BALB/c,通过摘除小鼠眼球,眼眶采血,得到相应的抗原内影像抗体Ab2β[4]。进行预实验摸索乳凝集素喂服剂量,并最终确定0.25 mg为最适剂量(无体重下降、精神不振和活动减少等中毒表现的最大剂量)。

1.2 *研究方法*

1.2.1 乳鼠感染HRV模型的建立 本研究选用7日龄清洁级昆明小鼠乳鼠,故造模前无需再次检测粪便中是否含有轮状病毒抗原或血清中是否含有轮状病毒抗体。采用随机数字表法将120只乳鼠分为A、B、C组及对照组,每组各30只。A、B、C 3组乳鼠灌胃50 μL HRV病毒悬液($TCID_{50}=10^{-5.2}$/100 μL)方式造模[5],其中B组在造模前连续7 d喂服含有Ab2β抗体的乳凝集素0.25 mg,C组在造模后连续7 d喂服含有Ab2β抗体的乳凝集素0.25 mg,对照组灌胃不含病毒的细胞培养液。

1.2.2 乳鼠腹泻程度评估 每日观察乳鼠粪便排泄情况,基于粪便的颜色和形态对粪便进行评分:正常粪便(1分),淡黄色软便(2分),淡黄色稀便(3分),水样便(4分),评分≥2分即为腹泻[6-7]。乳鼠腹泻程度=粪便分数之和/粪便总数。

1.2.3 酶联免疫分析法(enzyme linked immunosorbent assay, ELISA)检测乳鼠粪便中HRV抗原含量 感染后7 d内每日均采用ELISA法检测乳鼠粪便中HRV抗原并比较,记录乳鼠感染前,感染后1、3、5、7 d时体重。使用轮状病毒抗原检测试剂盒对各组乳鼠粪便中HRV抗原含量进行检测,按试剂盒说明书进行操作,具体步骤如下:取适量粪便于1.5 mL EP管中,加入200 μL生理盐水涡旋震荡混匀,取上清液50 μL加入反应孔中;每孔加酶结合物50 μL,室温下避光孵育10 min;吸去反应孔内液体,加入50 μL洗液,重复该步骤10次;吸尽反应孔内液体,加入显色剂A、B各50 μL,室温下避光孵育10~15 min,酶标仪读取实验结果。

1.2.4　免疫组化检测乳鼠小肠组织细胞间粘附分子1(intercellular adhesion molecule-1, ICAM-1)的表达情况　感染后7 d完成各项检测后处死乳鼠,并采用免疫组化法检测感染后7 d各组乳鼠小肠中ICAM-1的表达情况,获取标本后采用切片SABC法制备,并在显微镜下观察切片,着色为棕黄色者为阳性,并利用Optimas数字彩色图像显微分析软件分析处理测定棕黄色颗粒的灰度值,软件默认黑色为0,白色为255,取其平均值作为ICAM-1表达灰度值。

1.3　统计学分析　采用SPSS 19.0统计分析软件进行数据分析,结果以$\bar{x}\pm s$表示。多样本均数间比较采用One-way ANOVA检验,组间两两比较采用Student-Newman-Keuls检验,以$P<0.05$为差异有统计学意义。

2　结　果

2.1　各组新生乳鼠不同时间点腹泻严重程度比较　对照组新生乳鼠各时间点均未非发生腹泻,A、B、C 3组乳鼠在HRV感染1 d后出现腹泻症状(评分≥2分),A组HRV感染后1~5 d时腹泻严重程度明显高于对照组(t=3.519、4.157、5.746、4.003、3.549,$P<0.05$),且B、C组腹泻程度在HRV感染后2~4 d低于A组,差异有统计学意义($P<0.05$),见表1。

2.2　各组新生乳鼠各时间点体重情况　各组乳鼠感染前、感染后1 d体重比较,差异无统计学意义($P>0.05$);感染后1、3、5、7 d各时相A组乳鼠体重均低于对照组(t=3.062、3.516、4.198、5.985,$P<0.05$),而B、C组乳鼠在HRV感染后3、5、7 d体重高于A组,差异有统计学意义(t_{B-A}=3.112、4.516、5.036,t_{C-A}=3.547、4.806、5.517,$P<0.05$),B、C组乳鼠在HRV感染后各时间点体重比较,差异均无统计学意义(t=1.025、0.841、0.574、0.915,$P>0.05$),见表2。

2.3　各组新生乳鼠不同时间点粪便中HRV抗原含量比较　各组乳鼠在HRV感染1 d后粪便中检测出病毒抗原,A组感染后各时间点HRV抗原含量均高于对照组(t=3.065、3.519、3.881、3.617、3.391、3.258、3.012,$P<0.05$),而B、C组乳鼠粪便中HRV抗原含量在HRV感染后1~5 d低于A组,差异有统计学意义($P<0.05$),B、C组各时间点腹泻程度和HRV抗原含量比较,差异均无统计学意义(t=0.716、0.524、0.398、0.746、0.548、0.560、0.715,$P>0.05$),见表3。

2.4　各组新生乳鼠感染后7 d小肠组织ICAM-1表达情况　A、B、C 3组乳鼠在HRV感染7 d后,小肠组织ICAM-1表达细胞数高于对照组,差异有统计学意义($P<0.05$),B、C组ICAM-1表达细胞数及灰度值低于A组,差异有统计学意义($P<0.05$),见表4。免疫组化结果见图1。

表1　各组新生乳鼠不同时间点腹泻严重程度评分比较

Table 1　Comparison of the severity score of diarrhea at different time points in newborn mice in each group

组别Group	感染后1 d 1 d post infection	感染后2 d 2 d post infection	感染后3 d 3 d post infection	感染后4 d 4 d post infection	感染后5 d 5 d post infection	感染后6 d 6 d post infection	感染后7 d 7 d post infection
对照Control	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
A	1.83±0.38	2.70±1.02	3.50±0.68	2.50±0.90	2.03±0.96	1.47±0.63	1.17±0.38
B	1.56±0.50	2.13±0.82[a]	2.43±1.14[b]	1.63±0.56[b]	1.60±0.62	1.27±0.52	1.00±0.00
C	1.67±0.45	2.07±0.83[a]	2.53±1.04[b]	1.73±0.58[b]	1.63±0.67	1.30±0.53	1.00±0.00
F	3.849	5.847	4.637	4.056	3.912	1.256	0.419
P	0.014	0.005	0.009	0.011	0.013	0.179	0.452

注:a.与A组比较,$P<0.05$;b.与A组比较,$P<0.01$。Note: a. Compared with group A, $P<0.05$; b. Compared with group A, $P<0.01$.

表2　各组新生乳鼠各时间点体重比较　g

Table 2　Comparison of weight of newborn mice in each group at each time point　g

组别Group	感染前Pre-infection	感染后1 d 1 d post infection	感染后3 d 3 d post infection	感染后5 d 5 d post infection	感染后7 d 7 d post infection
对照组Control	16.83±2.56	19.65±2.83	20.64±1.79	22.90±2.25	25.58±2.43
A	17.03±1.17	17.81±1.96	18.34±2.40	19.60±2.55	21.54±2.03
B	17.25±1.79	18.21±2.33	19.85±2.27[a]	22.23±2.68[b]	24.92±2.49[b]
C	17.62±2.95	17.96±2.13	20.29±1.47[b]	21.93±2.35[b]	25.13±1.90[b]
F	0.419	3.391	4.082	3.547	3.846
P	0.337	0.042	0.038	0.044	0.040

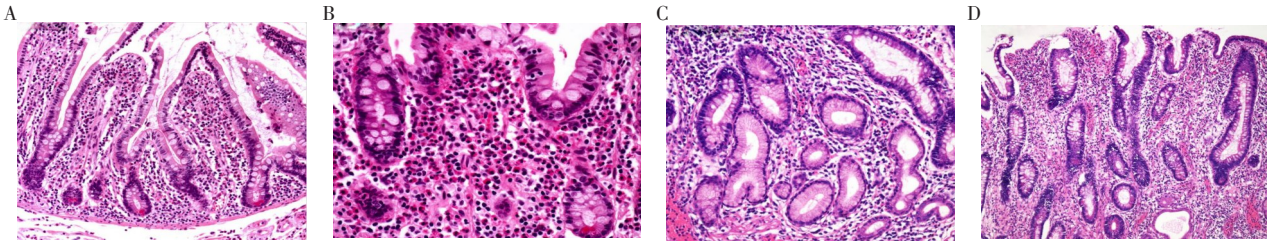
注:a.与A组比较,$P<0.05$;b.与A组比较,$P<0.01$。Note: a. Compared with group A, $P<0.05$; b. Compared with group A, $P<0.01$.

表3 各组新生乳鼠各时间点HRV检测含量比较 μg/mL

Table 3 Comparison of HRV detection content of newborn mice in each group at each time point μg/mL

组别 Group	感染后1 d 1 d post infection	感染后2 d 2 d post infection	感染后3 d 3 d post infection	感染后4 d 4 d post infection	感染后5 d 5 d post infection	感染后6 d 6 d post infection	感染后7 d 7 d post infection
对照 Control	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00
A	2.80±0.46	3.07±0.39	3.61±0.36	3.26±0.35	2.89±0.57	2.03±0.28	1.53±0.20
B	2.06±0.37[a]	2.47±0.54[a]	2.62±0.31[a]	2.10±0.40[a]	1.30±0.19[a]	1.00±0.00	1.00±0.00
C	2.30±0.34[a]	2.65±0.44[a]	2.79±0.37[a]	2.02±0.24[a]	1.26±0.17[a]	1.00±0.00	1.00±0.00
F	3.819	4.513	4.819	4.720	4.216	2.756	2.064
P	0.025	0.012	0.010	0.011	0.018	0.042	0.091

注：a. 与A比较，$P<0.01$。Note: a. Compared with group A, $P<0.01$.



注：A. 对照组；B. A组感染后7 d；C. B组感染后7 d；D. C组感染后7 d。Note: A. Control group; B. 7 days after infection in Group A; C. 7 days after infection in Group B; D. 7 days after infection in Group C.

图1 各组小肠ICAM-1免疫组化结果（SABC，×200）

Fig. 1 Immunohistochemical results of ICAM-1 in small intestine of groups (SABC, ×200)

表4 各组新生乳鼠感染后7 d小肠组织ICAM-1表达细胞数及灰度值比较

Table 4 Comparison of ICAM-1 expression cells and gray value in small intestine of newborn mice in each group 7 d after infection

组别 Group	ICAM-1表达细胞数 No. of cells expressing ICAM-1/%	灰度值 Gray value
对照组 Control	38.90±3.77	102.00±2.21
A	53.93±4.81	108.91±3.54
B	43.57±4.14[a]	100.88±2.32[a]
C	43.40±3.84[a]	101.14±2.48[a]
F	8.419	4.185
P	0.001	0.013

注：a. 与A组比较，$P<0.01$。Note: a. Compared with group A, $P<0.01$.

3 讨 论

HRV主要感染小肠上皮细胞引起细胞损伤，导致婴幼儿出现发热、呕吐和腹泻等临床症状，严重者可侵犯肠道外器官，导致轮状病毒血症，轮状病毒中枢神经系统损害等[8]。HRV主要流行于夏秋冬季，有明显的季节性。目前国内外疫苗均为针对轮状病毒衣壳蛋白的单价或多价疫苗，美国第1个轮状病毒的恒河猴链疫苗RRV虽然得到了政府许可，但应用后发生了发热和肠套叠等副反应[9]；中国兰州羊链轮状病毒疫苗是中国唯一被政府批准的轮状病毒疫苗，但其作用具有地区局限性；世界范围内公认有效并被广泛应用的减毒单价疫苗G1P8株（Rotarix）和人牛重组G1、G2、G3、G4和P8株（RotaTeq）疫苗，其有效性也存在地区性差异以及肠套叠发生率观察结果不完全一致的问题[10]，而DNA疫苗虽然可以诱导特异性的抗体和细胞毒性T淋巴细胞产生，但完整的DNA疫苗作用于宿主的染色体，可导致恶性肿瘤发病风险提升，因此研究安全、有效的小儿轮状病毒感染预防与治疗措施具有重要的临床价值[11-12]。

母乳中的乳凝集素在降低HRV感染导致的腹泻病发率和改善相关临床症状方面起着重要的作用，乳凝集素是由34个氨基酸组成的一种粘附在乳脂肪小球膜表面的具有免疫源性的糖蛋白，在其N端有两个上表皮生长因素（epidermal growth factor, EGF）样结构域，其中一个EGF样结构域是由精氨酸、甘氨酸和天冬氨酸组成的序列模体，能与细胞上表达的整合素相交联介导一系列免疫反应，具有促进肠上皮细胞迁移、肠粘膜修复的作用[13-14]，从而维持肠上皮的免疫微环境的稳定，而由孔凝集经单克隆抗体制备和免疫动物而来的抗体Ab2β，具有自体和异体免疫原性的抗原决定膜，能诱导不同种机体产生抗原特异性的免疫应答[15]，可增强其与轮状病毒交联的结合以及与小肠上皮细胞表面的N-乙酰神经氨酸唾液酸受体的结合，从而可以抑制轮状病毒的感染，同时内影像的抗独特型抗体Ab2β可刺激机体产生抗体和固有免疫细胞相关细胞增殖分化，进而提升轮状病毒感染腹泻的预防及治疗效果[16]。本研究结果显示，A、B、C 3组在

HRV感染后1 d均出现腹泻症状，但B、C组HRV检测值明显低于A组，且B、C组各时相HRV检测值比较差异无统计学意义，各组新生乳鼠感染前、感染后1 d时体重比较差异无统计学意义，而B、C组乳鼠各时相体重与对照组比较差异无统计学意义，但A组感染后3、5、7 d时体重明显低于对照组、B组、C组，感染后7 d A、B、C 3组ICAM-1表达细胞数明显高于对照组，且B、C组ICAM表达细胞数明显低于对照组。表明乳凝集素内抗体Ab2β能够有效预防及治疗轮状病毒感染，并对改善感染病情、体重等方面具有积极作用。

在正常肠组织中ICAM-1表达细胞数往往较低，而在肠道炎症的白细胞运动、募集中具有重要的影响作用，在肠道反应急性期可出现ICAM-1表达的显著上调，有研究发现ICAM-1缺失的肠损伤小鼠死亡率明显降低，且肠道炎症浸润与损伤程度较轻[17-18]，表明ICAM-1的表达对肠道损伤与炎性浸润的发生发展中具有重要的作用，而乳凝集素中独特抗体具备的促进肠上皮细胞迁移以及肠黏膜修复作用，能够有效抑制肠道炎性反应的进一步加重，其具体作用机制可能与对ICAM-1表达的抑制有关[19-20]。

综上所述，乳凝集素抗独特型抗体Ab2β对新生乳鼠人轮状病毒感染具有很好的预防与治疗作用，可显著改善腹泻症状、减降低HRV病毒载量，其具体作用机制可能与抑制小肠组织ICAM-1的表达有关。

伦理审查与知情同意　本研究获得合肥市第三人民医院伦理委员会批准（伦理号：2022LLW021），安徽医科大学生物医学研究所伦理委员会实验动物使用许可（LLSC20200832）

利益冲突声明　所有作者声明不存在利益冲突

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收稿日期:2022-12-05 **编辑**:黄艳

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收稿日期:2022-10-25 **编辑**:朱学义 王佳燕