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Detection of minus-stranded GBV-C/HGV RNA in the serum, liver and peripheral blood mononuclear cells in patients with hepatitis

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Abstract Objective: To investigate the presence of plus- and minus-stranded HGV RNA in the serum, liver and peripheral blood mononuclear cells (PBMC). Methods: Plus- and minus-stranded HGV RNA was detected with reverse-transcription nested polymerase chain reaction in 27 patients with hepatitis. Results: Plus-stranded HGV RNA was found in 21 cases in serum 10 cases in liver sample and 7 cases in PBMC. No minus stranded HGV RNA was found in serum and PBMC. Minus-stranded HGV RNA was detectal in 2 cases infected only with GBV-C/HGV in the liver sample. Plus-stranded HGV RNA was found in 10 of 21 liver samples from the patients with positive detection of HGV RNA in the serum and 2 of minus-stranded positive samples. No minus-stranded HGV RNA was found in the serum and PBMC. Conclusion: GBV-C/HGV is a hepatotropic virus that replicates in the human liver.

Key words GB virus C; hepatitis G virus; RT-nPCR; viral replication

ELISA 法初筛献血员 anti-HIV(1/2 型)初探

Priliminary investigation on the early screening of anti-HIV (1/2) of blood donors by ELISA

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我科用4种不同厂家生产的酶联免疫吸附(ELISA)试剂2 277人次献血员初筛anti-HIV(1/2型)抗体,结果报道如下。

1 材料与方法

1.1 标本的采集与处理

于献血前采集小标本,空腹肘静脉血2 ml,不抗凝;采血后留取2 ml不抗凝标本。标本充分吸收后,离心得到血清,以消除纤维蛋白的干扰。检测在4 h内进行完毕。

1.2 方法

1.2.1 试剂 ELISA法试剂:卫生部规定anti-HIV检测试剂必须使用经卫生部药品生物制品检定所每批检定合格的产品(《关于对四种体外免疫诊断试剂生产、销售、使用、管理的补充通知》卫药发(1995)第32号文)。我们使用的4种ELISA试剂分别由:上海实业科华生物技术有限公司、珠海丽珠医药集团股份有限公司、华美生物工程公司、厦门新创科技有限公司所生产。试剂盒经卫生部药品生物制品检定所每批检定都合格。

每次实验都设空白对照、阴性对照、anti-HIV(1型)、anti-HIV(2型)阳性对照,以消除实验系统误差。实验由专人操作、固定加样器、水浴箱温度恒定37℃。具体操作按试剂盒说明书进行。

1.2.2 仪器 微量加样器定期进行校正,酶标仪使用DG3022A型单孔酶标仪。D_λ值测定取单波长450 mm。

结果判定:D_λ值≥cut off值为阳性,否则为阴性。

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A study of superantigen-induced T-cell apoptosis using establishment of short-term superantigen-specific T cell line from human peripheral blood*

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Abstract Objective: To establish the short-term superantigen-specific T cell line from human peripheral blood and study the superantigen-induced T-cell apoptosis. Methods: The short-term SEA-reactive T-cell line was established by simulation of PBMC 10^6 with 0.5～5 μg/ml SEA for 3 d and followed by expansion of cells in rIL-2 (200 U/ml) for 2 weeks. The apoptosis of the cell line was detected with both FCM and DNA electrophoresis analysis. Results: The short-term SEA-reactive T-cell line was established by 0.5～5 μg/ml SEA and 200 U/ml rIL-2 from PBMC and the cell line could be maintained for 40 d *in vitro*. Meanwhile, it was found that the apoptosis appeared after the second stimulation of the SEA-reactive T cells with SEA 1 ug/ml. Conclusion: The short-term superantigen-specific T cell line from human peripheral blood can be established by stimulation of SAg and rIL-2. T-cell apoptosis appears after the second stimulation with SEA in the anergy induced by superantigen.

Key words superantigen; T cell; apoptosis; cell line; PBMC

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2 结果

4种 ELISA 试剂检测 anti-HIV(1/2型)抗体结果见表1。

表1 4种 ELISA 试剂检测 anti-HIV (1/2型)抗体结果

试剂	实验总人次	阳性例数	比例
上海实业科华生物技术有限公司试剂	832	7	0.0084
华美生物工程公司试剂	428	4	0.0093
厦门新创科技有限公司试剂	273	2	0.0073
珠海丽珠医药集团股份有限公司试剂	744	40	0.0538
总 体	2277	53	0.0233

3 讨论

ELISA 法初筛献血员 anti-HIV 时，凡献血员 ELISA 法检测 anti-HIV(1/2型)阳性结果者要求进行双孔复试。复试阴性的可以确定为阴性，复试阳性的要求送 HIV 确认实验室进一步确认，现常用免疫印迹法(WB)和明胶颗粒法(PA)两种方法进行检测[1]。确认试验阴性的血制品可以应用临床，同时首次 ELISA 法阳性结果被判定为假阳性。本结果中53名初筛阳性者中50人双孔复试结果为阴性，3人双孔复试结果阳性，送交重庆市防疫站 HIV 确认实验室，经确认为阴性，故该53份阳性结果全都是假阳性。

我们在进行 ELISA 法初筛献血员的同时，对 ELISA 法首次试验阳性标本采用免疫金标记斑点渗滤法(总后勤部卫生部提供试剂)检测。结果与 ELISA 法双孔复试结果相符。

目前临床应用的 anti-HIV ELISA 法试剂灵敏度较高，但特异性较差，易出现假阳性结果。这可能缘于：加样方式影响 ELISA 法检测结果；加样时吹吸血清产生气泡；洗板不彻底；扣干不完全孔内残留液体过多等实验操作不标准因素。另一个最主要的原因是：献血员血清中所含有的高浓度的非特异性 IgG[2,3]。献血员血清中受检的特异性 IgG 只占总 IgG 中的一小部分，IgG 对聚苯乙烯有较强的吸附性能，抗体或蛋白质抗原吸附其上后保留原来的免疫活性。ELISA 板的制作过程中，为了增加聚苯乙烯对蛋白质尤其是免疫球蛋白的吸附性能，往往使用射线照射聚苯乙烯后再包被制板。如果包被过程中聚苯乙烯表面没有被蛋白质完全覆盖，或封闭过程不完全，实验时加入的血清标本中的免疫球蛋白甚至酶结合物中的蛋白质部分地吸附到包被抗原的表面上，从而使本底增加，出现假阳性[2,3]。

关键词 献血员；ELISA；HIV

中图法分类号 R446.61；R512.91

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