УДК: 616-089.843
Материал поступил в редакцию: 11-07-2014
Материал принят к печати: 28-07-2014

Development of adoptive immunotherapy technology in post-transplantation period

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After liver transplantation due to recurrent HCV infection, recurrence of hepatocellular carcinoma is common. It leads to graft loss and patient's death. Adoptive immunotherapy based on natural killers has huge potential in prevention of hepatitis C recurrence and subsequent hepatocellular carcinoma development.

Keywords: natural killers, immunotherapy, graft, hepatocellular carcinoma.

J Clin Med Kaz 2014; 2(32): 73-75

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ТРАНСПЛАНТАЦИЯДАН КЕЙІНГІ КЕЗЕҢДЕ АДОПТИВТІ ИММУНОТЕРАПИЯСЫН ЕНГІЗУ ТЕХНОЛОГИЯСЫ

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Бауыр трансплантациясынан кейін болатын С гепатитінің вирусімен қайта жұғу салдарынан дамитын гепатоцеллюлярлі карцинома, трансплантаттың жарамасыз болып, науқас өліміне әкеледі. Натурал киллерлерге бағытталған адоптивті иммунотерапия С гепатитінің қайта жұғуын және гепатоцеллюлярлі карцинома дамуының алдын-ала шарасы ретінде үлкен маңызы бар.

Маңызды сөздер: натурал киллерлер, иммунотерапия, трансплантат, гепатоцеллюлярлі карцинома

ОСВОЕНИЕ ТЕХНОЛОГИИ АДОПТИВНОЙ ИММУНОТЕРАПИИ В ПОСТ-ТРАНСПЛАНТАЦИОННЫЙ ПЕРИОД

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После трансплантации печени из-за рецидивирующего заражения ВГС часто происходит рецидив гепатоцеллюлярной карциномы, что ведет к потере трансплантата и смерти пациента. Адоптивная иммунотерапия на основе натуральных киллеров имеет большой потенциал для профилактики рецидива гепатита С и повторного развития гепатоцеллюлярной карциномы.

Ключевые слова: натуральные киллеры, иммунотерапия, трансплантат, гепатоцеллюлярная карцинома

Application for natural killers in immunotherapy for tumors' proliferation suppression and prevention of recurrent hepatocellular carcinoma after liver transplantation.

Liver failure and hepatocellular carcinoma (HCC) as a result of hepatitis C are considered as the most common indications for liver transplantation. Recurrent hepatitis C virus (HCV) infection of allograft is pervasive and occurs immediately after liver transplantation (LT) and associated with accelerated progression to liver cirrhosis, graft loss and death. It happens because of suppression of host-effector immune responses, which are responsible for HCV replication control, suggesting that immunosuppressive environment plays major role in fast progression of recurrent HCV infection after LT.

Furthermore, immunosuppressive conditions, described above, are thought to be one of the main cause for increase of cancer recurrence incidence after LT among patients with HCC

At the present time adjuvant immunotherapy technology can be applied for recurrent HCC prevention

after LT; this immunotherapy includes intravenous infusion of activated natural killer cells (NK cells). Forasmuch immunosuppressive regimen, used after LT, reduces adaptive immunity components, but efficiently increases innate cell immunity components and NK cells response, which plays pivotal role in innate immunity, can become promising immunotherapeutic approach.

Natural killers are cytotoxic lymphocytes and are fundamental component of innate immunity. NK cells are capable to recognize and destroy tumor cells as well as cells infected by viruses or bacteria. By modulating of dendritic cells functions and T-cells antigen-specific response control, natural killers play important role in adaptive immunity. NK cells' ability to kill tumor cells makes them interesting for immunotherapy. Furthermore, unlike traditional therapeutic approaches, NK cells based therapy more efficient in metastasis treatment. NK cells are widespread in organism, they are detected in spleen, liver, bone marrow, peripheral blood and lymph nodes. In human organism NK cells play important role in protection from HIV, herpes, HBV, HCV and other viral infections and that became the main reason for research in post-transplantation period application. Natural killers are first line defense against tumor proliferation.

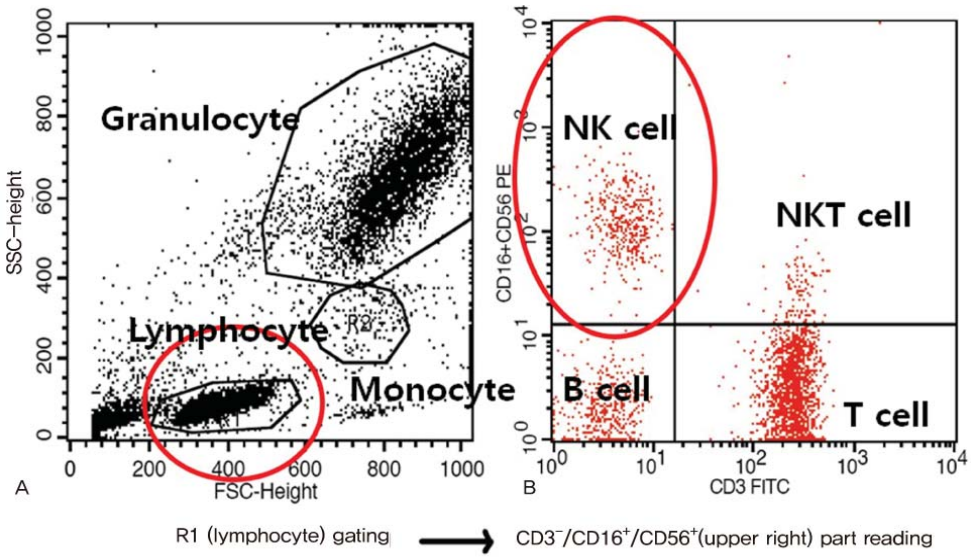
During the research following materials were use: peripheral blood (with heparin 1 ml/20 ml); ficoll solution; 15 мл ml polystyrol cone tubes; 15 ml polypropylene cone tubes; 1× PBSS; 50 ml centrifuge tubes.

PERIPHERAL BLOOD SAMPLE COLLECTION

In a research we used heparinized blood. Sample tubes were centrifuged at 2500 rpm during the 5 minutes. Plasma is separated and discarded , cell suspense is filled by 1x PBS up to 20 ml. Diluted blood is layered by 10 ml per 5 ml of ficcol solution. Tubes are centrifuged at 1500 rpm during the 30 minutes. After centrifuge interphase ring with mononuclear cells is collected into separate tube. Suspension of mononuclear cells is washed by RPMI medium at 2000 rpm during the 5 minutes. Cells are resuspended in 1 ml of cultural medium. Cells are counted in hemocytometer by 0,1% trypan blue staining.

FLOW CYTOMETRY

Flow cytometry assay was performed by using a FACS-Calibur dual-laser cytometer (BD Biosciences). The following mAbs were used for the surface staining of the lymphocytes: FITC-conjugated anti-CD3 mAb (clone HIT3a; BD Biosciences — Pharmingen); PE-conjugated anti-CD56 mAb (clone B159; BD Biosciences — Pharmingen); and biotinylated anti-TRAIL (biotin-conjugated anti-TRAIL) mAb (clone RIK-2; eBioscience). The biotinylated mAb was visualized using APC-streptavidin (BD Biosciences — Pharmingen). Dead cells identified by light scatter and propidium iodide staining were excluded from the analysis. IFN-γ production in the lymphocytes was measured by a combination of cell surface and cytoplasmic mAb staining and subsequent flow cytometric assay, as mentioned previously.



CELL CULTURE

Cell cultivation is carried out in X-VIVO medium with 10% FBS, IL-2 and anti CD4 with concentration of CO2 5% and 37ºC temperature.

After liver transplantation to patients with HCV, viral burden inevitably surpasses level before transplantation. This is because of suppression of host-effector immune respone

components, that is controlled by immunosuppressive drugs, used to prevent liver graft rejection. There is an acceptable immunotherapy approach by using lymphocytes, extracted from allograft liver perfusate, including NK/NKT cells, which produce sustain anti-HCV immune response in HCV infected liver, after transplantation and despite to immunosuppression. This therapy This therapy is performed as follows:3 days after allotransplantation lymphocytes, coated by IL-2, CD-3 and OKT3 specific mAbs, are injected intravenously. This procedure prevents replication of HCV in infected liver.

REFERENCES

1. Possibility of adoptive immunotherapy with peripheral blood-derived CD3□CD56+ and CD3+CD56+ cells for inducing antihepatocellular carcinoma and antihepatitis C virus activity. Doskali M, Tanaka Y, Ohira M, Ishiyama K, Tashiro H, Chayama K, Ohdan H.
2. Adoptive immunotherapy with liver allograft–derived lymphocytes induces anti-HCV activity after liver transplantation in humans and humanized mice. Masahiro Ohira, Kohei Ishiyama, Yuka Tanaka, Marlen Doskali, Yuka Igarashi, Hirotaka Tashiro, Nobuhiko Hiraga, Michio Imamura, Naoya Sakamoto,Toshimasa Asahara, Kazuaki Chayama and Hideki Ohdan // J Clin Invest. 2009.-Vol.119(11).-P.3226–3235.