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CD26/DPPIV and response to hepatitis B vaccination

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Abstract

The prevention of hepatitis B is important, since it is responsible for significant morbidity and mortality around the world. Unfortunately, hepatitis B vaccine does not always induce protective immunity. The lack of immune response to vaccine (non-responders) can depend on individual characteristics.

The objective of this study was to correlate the CD26/DPPIV cellular expression and DPPIV serum activity with HBV vaccine response and its possible role as an indicator of immune competence acquisition. We also determined the cellular expression of CD3, CD19, CD56 and CD25 in peripheral blood T lymphocytes.

Blood samples were obtained from 28 healthy human volunteers who were enrolled with a vaccination program. There were "responders" (RM = 13) and "non-responders" (NRM = 15), after vaccination. The lymphocyte populations were identified by flow cytometry. DPPIV serum activity was measured fluorimetrically.

CD26 expression in responders (55.9 ± 7.7%) versus in non-responders (51.9 ± 7.0%) did not show a significant difference. The DPPIV serum activity in responders compared to in non-responder subgroup (59.9 ± 8.4/50.3 ± 10.6 U/L) showed, however, a significant difference ($P < 0.05$). The expression of CD3, CD19 and CD56 on peripheral lymphocytes was similar between responders and non-responders. The expression of CD3CD26 (52.2 ± 8.6%) and CD3CD25 (10.9 ± 3.8%) in responders versus the expression of CD3CD26 (48.0 ± 5.7%) and CD3CD25 (8 ± 4.6%) in non-responders did not show statistically significant difference.

CD25 referred as a marker of T lymphocyte activation was increased in responders (15.8 ± 4.5%) versus in non-responders (10.1 ± 4.8%), showing a significant difference ($P = 0.003$). It was, however, impossible to demonstrate an increase in CD3CD25 and CD3CD26 in the responder subgroup. This suggests that different lymphocyte subsets other than T cells are implicated in the response to hepatitis B vaccination.

Keywords: Hepatitis B; Vaccination response; Dipeptidyl peptidase IV (DPPIV); CD26; Immune response

1. Introduction

Hepatitis B is one of the most important infectious diseases around the world. Its importance is due to the enormous number of infected persons, about two billion. It is also due to its subsequent complications such as fulminant and fatal acute hepatitis, chronic liver disease with cirrhosis and even hepatocellular carcinoma. The chronic carriers constitute the main human reservoir of hepatitis B [1,2]. Groups with high rates of hepatitis B virus (HBV) infection include also healthcare workers daily exposed to blood and other body fluids. In these particularly exposed groups, vaccination programs are indicated, since the hospital-wide hepatitis B immunisation programme helps to raise the immune status of the staff, and to reduce the costs of prophylactic usage of hepatitis B immunoglobulin.

The recombinant hepatitis B virus vaccine has been used for more than a decade. It consists in non-glycosylated HbsAg particles, but it is otherwise indistinguishable from natural HbsAg. It is comparable, in immunogenecity, protective efficacy and safety to the first generation plasma-derived vaccine [1,3].

Unfortunately, 2–10% of healthy and immunocompetent adults do not respond to vaccination with the production of protective levels of anti-HBS antibody. This fact could be related to a diversity of factors such as:

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sex, age, genetic factors, and some environmental factors [4].

Nevertheless, as hepatitis B is a preventable disease by immunization, the most efficient way to prevent it, is a vaccination program. It is agreed that a universal hepatitis B vaccination should be encouraged in order to reduce the morbidity and the mortality attributable to liver disease and its complications [5,6].

Traditionally HBV vaccination response markers have been the anti-Hbs antibodies serum titters (>100 U/L, responders; <10 U/L, non-responders). The knowledge of the mechanisms by which the immune response can be induced is important, and so is the knowledge of new peripheral blood cell markers, which should give an overview of the immune system pathways.

Dipeptidyl peptidase IV (DPPIV), a membrane bound exopeptidase, has been identified as the surface antigen CD26. Like several other surface enzymes, CD26/DPPIV is expressed on a variety of tissues and cell types, including the T cells, in particular in memory T lymphocytes, as well as on the endothelial and epithelial cells [7].

CD26/DPPIV is a multifunctional molecule that interferes with many immune functions, both in vitro and in vivo. In addition to its membrane-associated form, CD26/DPPIV is also present as a soluble exopeptidase in various body fluids, such as plasma, serum and urine [8–21].

DPPIV has an unique aminopeptidase activity. It cleaves dipeptides from the NH2-terminus of proteins, having a proline, a hydroxiproline or an alanine residue at the penultimate position, with the highest efficiency observed with proline residues [13,19,22–25].

Independently of its peptidase activity, CD26/DPPIV is associated to other molecules on the cell surface. For instance, on T lymphocytes, it is associated with CD45, a cell surface expressed phosphotyrosine phosphatase, which is involved in signal transduction. It has been shown to be the main receptor of adenosine deaminase of the T-cell surface, thus protecting the cell from adenosine mediated inhibition of proliferation. In addition, it has also been proposed that CD26 is involved in the pathophysiology of the acquired immune deficiency syndrome (AIDS). It is clear that CD26/DPPIV is involved in multiple functional activities related with immune response. [10–12,14,15,22,26–37].

Both features of CD26/DPPIV, as a membrane antigen and as a peptidase, contribute to the co-stimulation of CD26 in T-cell activation events. The role of CD26/DPPIV in these events is, however, unclear [10,14,38–40].

In view of the established relevance of CD26/DPPIV as a T-cell activation marker our aim was to clarify whether its expression on T cells would change after hepatitis B vaccination and, according to the vaccination response, what differences are observed [8]. Therefore, we studied the expression of CD26 in the peripheral blood T lymphocytes and serum DPPIV activity. The main lymphocyte populations (CD3, CD19 and CD56), were also studied for comparison to see the expression rate of CD26 in T lymphocytes (CD3) and also of CD25, a known marker of T lymphocyte activation.

2. Materials and methods

2.1. Subjects and blood sampling

Healthy volunteer healthcare workers ($M = 28$) at the University Hospital of Coimbra (HUC) who were enrolled in an anti-HBV vaccination program, proposed by the medical service of the hospital, participated in this study.

After the vaccination program, individuals were grouped as: “responders” (R), anti-Hbs >100 UI/L ($n = 13$) and “non-responders” (NR), anti-Hbs <10 UI/L ($n = 15$).

Peripheral blood samples (10 mL) were obtained by venous puncture. The samples were divided in two aliquots: 4 mL were collected with EDTA to perform flow cytometry analysis; 6 mL was collected without anticoagulant and allowed to clot at room temperature, centrifuged at 2000 × g, for 15 min, and the serum was removed and stored at −30 °C, until assayed.

2.2. Flow cytometry

We used monoclonal antibodies that were directly conjugated to one of the following fluorochromes: fluorescein isothiocyanate (FICT), phycoerythrin (PE), or phycoerythrin–cyanin 5.1 (PECy5).

Lymphocyte subsets were studied by two and three colour immunofluorescences with conjugated monoclonal antibodies: anti-CD3 from Dako® (PECy5); anti-CD19 (Fitc) and anti-CD56 (PE) from Immunotech®, anti-CD26 (PE) and anti-CD25 (PE) from Immune Source®. Residual red blood cells were lysed with “Lysing Solution®” from Becton Dickinson®. Cells were resuspended in phosphate-buffered saline (PBS) until analysis.

Flow cytometry was performed using a FacsCalibur™ from Becton Dickinson, collecting 10,000 events by acquisition. The lymphocyte population was identified by light scattering and fluorescent properties, gated and analysed.

2.3. Serum DPPIV activity was measured by a fluorimetric assay previously described by Sharpé et al. [41]

DPPIV was recorded by the cleavage of the fluorogenic substrate Gly-Pro-4-Me-2-NA, Sigma–Aldrich Co. (St. Louis, 63178 Missouri, USA) releasing a highly fluorescent molecule: 4-Me-2-NA. For the substrate solution, 20 mmol/L, substrate was dissolved in 1 mL of DMSO. This solution was stored at 4 °C.

Standard solution, 4-Me-2-NA was acquired from Bachem Feinchemikalien AG, (Budendorf, Switzerland). The stock solution was 50 mmol/L 4-Me-2-NA in DMSO. Before use it was dissolved as required with stopping solution.

Incubation buffer was a 50 mmol/L Tris–HCl solution, pH 8.3, adjusted at room temperature, stored at 4 °C. Stopping solution was a 100 mmol/L citrate solution, pH 4.0, adjusted at room temperature and stored at the refrigerator at 4 °C. The fluorescence was measured with a JASCO FP-777 spectrofluorimeter, with a quartz cell, at 340 nm of excitation and at 425 nm of the emission wavelengths.

Serum DPPIV activity has been expressed in units/litre (U/L). One unit (U) of DPPIV activity was defined as the enzyme activity that produced 1 μmol of 4-Me-2-NA in 1 min under the reaction conditions.

2.4. Statistical analysis

Student's *t*-test was used to determine the statistical significance, considering a *P*-value of <0.05 being significant.

3. Results

CD26 expression in responders (55.9 ± 7.7%) versus in non-responders to hepatitis B vaccine (51.9 ± 7.0%) did not show a significant difference (Table 1, Fig. 1).

In the two studied subgroups, serum DPPIV activity was higher in R subgroup (59.9 ± 8.4 U/L) when compared to the enzyme activity in NR subgroup (50.3 ± 10.6 U/L) with a statistical significance ($P < 0.05$) (Table 2, Fig. 2).

Table 1
Monoclonal antibody markers—CD3, CD19, CD56, CD25, CD26, CD3CD26 and CD3CD25—in hepatitis B virus vaccinated, "non-responders" and "responders"

	Non-responders ($n = 15$)	Responders ($n = 13$)
CD3 (%)	79.6 ± 4.8	79.0 ± 7.7
CD19 (%)	9.6 ± 3.1	10.3 ± 3.8
CD56 (%)	10.7 ± 3.8	10.7 ± 4.8
CD25* (%)	10.1 ± 4.8	15.8 ± 4.5
CD26 (%)	51.9 ± 7.0	55.9 ± 7.7
CD3CD25 (%)	7.9 ± 4.6	10.9 ± 3.8
CD3CD26 (%)	49.0 ± 6.8	52.2 ± 8.6
CD3negCD26 (%)	2.8 ± 1.8	3.8 ± 1.6

Results are expressed in percentage of cells. CD26 is mainly expressed by CD3 T lymphocytes, since CD3neg cells only expressed ±2.8% of total CD26.
* $P = 0.003$.

Table 2
Protease DPPIV activity (U/L) in hepatitis B virus vaccinated group of healthcare workers grouped, according to the vaccination response, in "non-responders" and "responders"

	DPPIV (U/L)
Non-responders ($n = 15$)	50.3 ± 10.6
Responders ($n = 13$)	59.8 ± 8.4

The "responders" showed a higher, and statistically significant, DPPIV activity compared with "non-responders" DPPIV activity. Student's *t*-test: *$P < 0.05$.

The CD3, CD19 and CD56 expression in T lymphocytes was similar take in R and NR subgroups. The expression of CD3CD26 (52.2 ± 8.6%) and CD3CD25 (10.9 ± 3.8%) in responders versus (48.0 ± 5.7%) and (8 ± 4.6%), respectively, in non-responders, was not statistically significant.

In responders, the expression of CD25 (15.8 ± 4.5%) demonstrated a higher and significant difference when compared with non-responders (10.1 ± 4.8%) ($P = 0.003$).

4. Discussion

The vaccination is the most important way to protect the population against hepatitis B, since all are vulnerable due to its infectivity and its complications such as acute and fulminating hepatitis, cirrhosis and hepatocellular carcinoma [2,42].

Healthcare workers are daily in exposure risk to infected body fluids. That makes adequate hepatitis B immunisation of healthcare workers a priority.

The effectiveness of hepatitis B vaccines is well known. As previously mentioned traditionally serum titters of antibodies are classical markers of protective immunity to HBV infection. Unfortunately, a number of apparently healthy and immunocompetent adults (2–10%) are unable to achieve protective concentration of antibodies (>100 U/L) after vaccination. This suggests a defect in either B- or T-cell functions in non-responder individuals [43,44]. Nevertheless, antibody concentration only demonstrates a small part of the complexity of immune response, without any information about immune efficacy. The lack of response to hepatitis B vaccination remains a problem for those individuals who are directly in risk of hepatitis B infection.

When activated, T- and B-lymphocytes express a number of surface molecules that are absent or present in low concentrations in resting lymphocytes. Among these molecules are enzymes such as proteases like DPPIV, E.C.3.4.14.5 also known as CD26. It has become a subject of specific interest since it appears to have additional functions in T-cell activation as a co-stimulator molecule [14,39,45,46]. The presence of DPPIV in serum varies widely between individuals, and it is increased upon cell activation. Previous studies have shown roles for CD26 in T-cell activation. It is speculated that CD26/DPPIV is potentially important in the achievement of immunocompetence [7,47,48].

DPPIV cleaves several cytokines, which could change the characters of the local inflammatory response, affecting the differentiation of responding lymphocytes into helper, cytotoxic and regulatory cell populations [7]. The mechanism through which DPPIV influence T-cell functions has not, however, been completely established [49].

The increase of CD26 expression in T-lymphocyte and macrophage membranes has been linked to cell activation and development of immunological memory [49–51].

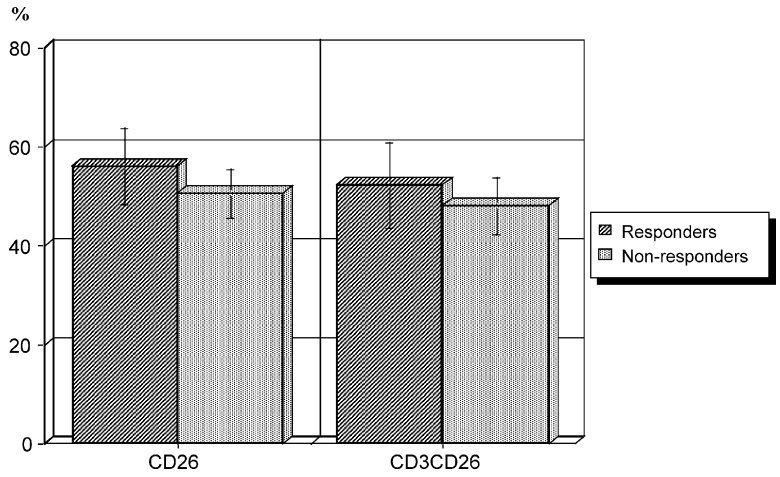

Fig. 1. CD26 expression in peripheral blood T lymphocytes, on responders and non-responders. Data in percentage of cells that expressed CD26 surface antigen.

The molecular mechanisms by which CD26 mediates the T-cell stimulation/activation mechanisms appear to be associated to the T-cell receptor (TCR), according to some authors, but the role of this molecule in the regulation of the immune response is only partly established [45,52].

According to other authors, the level of DPPIV activity is not directly related to the intensity of CD26 expression on the T cells [4,46]. Findings of others have led to the proposal that DPPIV/CD26 may be involved in the switching of the innate and acquired immune response [53].

Our present results showed that DPPIV serum activity was significantly increased in the responders after vaccination. In our opinion, these DPPIV increased serum levels may be related to successful activation of immune system and to the acquisition of immune efficiency. Other workers have published a significant decrease in T-cell proliferation, cytokine levels and antibody production under the influence of DPPIV specific inhibitors [14,54].

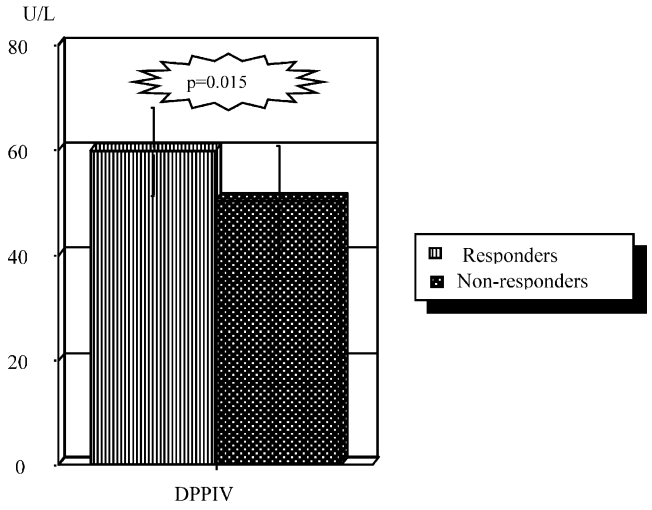

Fig. 2. Representation of DPPIV activity (U/L) in responders and non-responders hepatitis B virus, after vaccination.

The expression of CD26 on the peripheral blood T lymphocytes did not show a significant difference between the R and NR subgroups. The CD26 expression on CD3 lymphocytes, could be related to cells other than T lymphocytes, and the present result may be due to insufficient sample size.

The increased significant DPPIV activity and the referred modification of CD26 expression, in the responder subgroup, might reflect an improvement in the individual immune status after vaccination. This is emphasised by the significant increase of CD25 (a classical marker of T lymphocyte activation) expression on R subgroup. This could render a greater capacity to react against infection.

It remains to be proved that antibody concentration is the best immunity marker of immune competence after hepatitis B vaccination as the immunological memory, among many others, are involved in the protection of vaccinated organisms.

Further studies, involving larger sample sizes and additional complementary markers of immune protection will probably answer some of the remaining questions that continue to challenge our knowledge of CD26/DPPIV intervention in HBV immunisation.

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