

Distribution of Circulating Immune Cells in Responder and Non-Responder Individuals to Hepatitis B Vaccine

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ABSTRACT

Unresponsiveness to hepatitis B surface antigen (HBsAg) has been shown to be associated with dysfunction of the presenting cells (APC) and defect in the specific B-lymphocyte and/or T-lymphocyte repertoires. Direct determination of the frequency of specific T-lymphocytes together with complementary analysis of the naive circulating immune cells could provide valuable information about the cellular basis of unresponsiveness to HBsAg. In this study, the phenotypic characteristics of peripheral blood mononuclear cells (PBMC) from healthy adult high-responders (n = 19), intermediate-responders (n = 11), low-responders (n = 9) and non-responders (n = 6) to recombinant hepatitis B vaccine were investigated and compared. The proportions of circulating B-lymphocytes (CD19⁺ cells), T-lymphocytes (CD3⁺ cells) and monocytes (CD14⁺) were similar in all groups of responder individuals (14%, 55-60% and 11-13%, respectively) compared to non-responders (16%, 64% and 9%, respectively). These results suggest that the cellular basis for the lack of response to HBsAg is not associated to a generalized deficiency of immune cells in the non-responder subjects, rather it may reflect a defect in HBsAg-specific B or T cells. *Iran. Biomed. J. 6 (1): 1-5, 2002*

Keywords: HBs antigen, anti-HBs antibody, hepatitis B vaccine, B-lymphocyte, T-lymphocyte

INTRODUCTION

Exposure of healthy adult individuals to hepatitis B virus (HBV) results in a protective antibody response in 90-95% of cases, associated with either asymptomatic or acute clinical course [1]. Vaccination with the major surface protein of HBV (HBsAg) has also been found to induce a protective antibody response in a similar proportion of the normal adult population and also in neonates and children [2, 3]. However, 1-10% of the vaccines displays inadequate antibody response following primary vaccination with triple doses of either plasma-derived or recombinant HB vaccine. These individuals remain at risk to infection with HBV [4].

Non-responsiveness to HBsAg has been shown to be associated with lack or defect in antigen presenting cell (APC)/T-lymphocytes interactions [5, 6], specific B-lymphocytes [7-10] and T-lymphocytes [11-13]. Direct determination of the frequency of specific B and T-lymphocytes has important implications for investigation of the immune response to different antigens and pathogens [10, 13]. Complementary determination

of the naive circulating immune cells could also provide valuable information to elucidate the cellular basis for lack of response to HB vaccination. In the present study, the frequency of B-lymphocytes, T-lymphocytes and monocytes was determined in peripheral blood of responder and non-responder normal individuals vaccinated with recombinant HB vaccine, by flow cytometry.

MATERIALS AND METHODS

Subjects and vaccination schedule. A total of 45 healthy adult individuals (34 males, 11 females, aged 17-61 years old) that were negative for anti-HBs antibody and anti-HBc antibodies and HBsAg were vaccinated with triple 20 µg doses of recombinant HB vaccine (Heberbiovac, S.A. Havana, Cuba) as previously described [14, 15]. Two to four weeks after completion of vaccination, the serum level of anti-HBs antibody and the frequency of immune cells in peripheral blood were determined [3, 12, 16].

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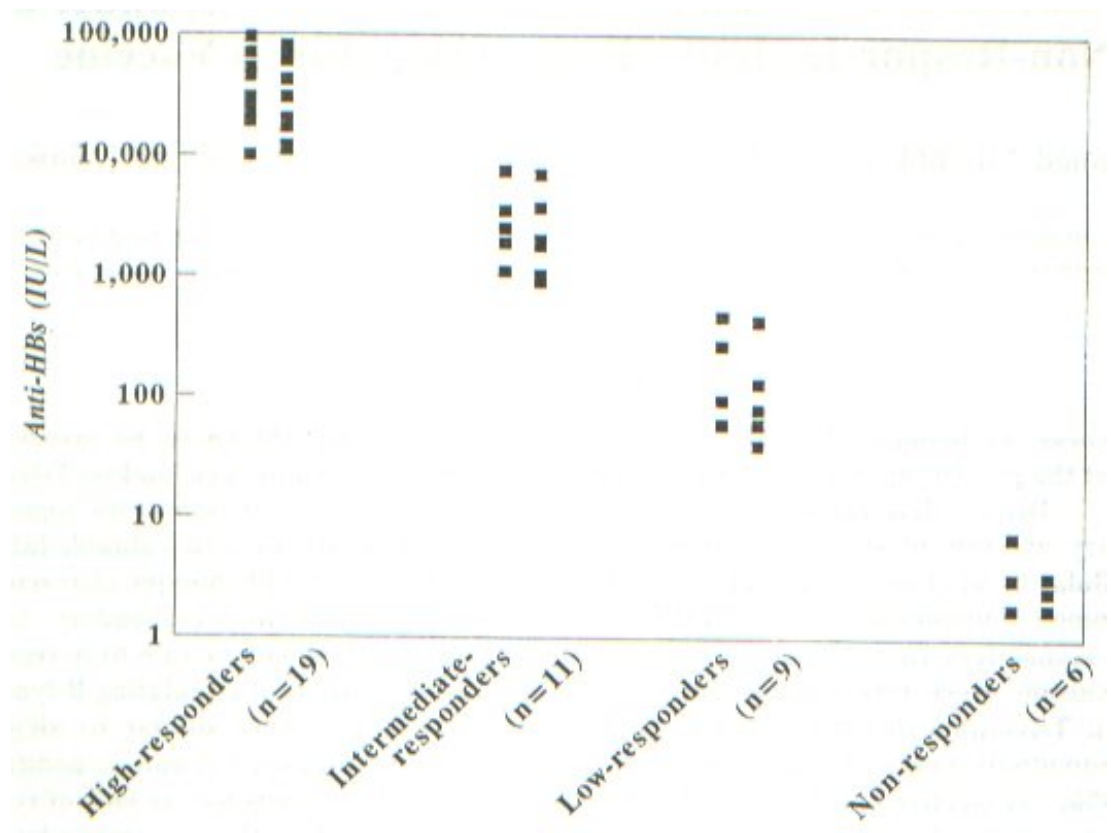


Fig. 1. Serum levels of anti-HBs antibody in responder and non-responder vaccines.

Detection of HBV markers. Anti-HBs and anti-HBc antibodies and HBsAg were detected by ELISA, using commercial kits (Behring, Germany). Anti-HBs antibody was quantitated in serum using appropriate dilutions of a positive sample with known concentration of the antibody expressed as IU/L provided by the manufacture.

Enumeration of immune cells in peripheral blood. Peripheral blood mononuclear cells (PBMC) ($0.5 - 1 \times 10^6$) were washed twice with cold PBS (pH 7.4, 0.15 M) and incubated with FITC conjugated mouse monoclonal anti-human CD3 (F 0818, DAKO, Denmark), anti-human CD14 (F 0844, DAKO, Denmark), anti-human CD19 (F 0768, DAKO, Denmark), and a isotype matched negative control in the dark at 4°C for 30 minutes. After incubation, the cells were washed twice with PBS supplemented with 2% bovine serum albumin (BSA) (Sigma, USA). The cells were then fixed in 1% paraformaldehyde solution (Sigma, USA) before analysis under fluorescence activated cell sorter (FACScan, Becton Dickinson, UK).

Statistical analysis. Comparison of variables was analyzed using the student's *t*-test and $p < 0.05$ was considered significant.

RESULTS

Measurement of anti-HBs antibody in serum. Anti-HBs antibody was measured in serum of all subjects before and after vaccination. Detectable level of anti-HBs was not identified before vaccination in any of the individuals. The results obtained after the completion of the vaccination are illustrated in Figure 1. Based on the titer of anti-HBs antibody, the vaccines were arbitrary classified into 3 groups: high-responders ($n = 19$) (anti-HBs antibody $> 10,000$ IU/L), intermediate-responders ($n = 11$) (anti-HBs antibody: $>500 < 10000$ IU/L), low-responders ($n = 9$) (anti-HBs antibody: $>100 < 500$ IU/L) and non-responders ($n = 6$) (anti-HBs antibody < 10 IU/L) [12, 17]. The mean titers of anti-HBs antibody were 40,000, 2338, 182 and 3.3 IU/L in high, intermediate, low and non-responders, respectively.

Enumeration of CD3⁺, CD19⁺ and CD14⁺ cells in PBMC of responder and non-responder individuals. Membrane expression of CD3, CD14 and CD19 in PBMC was tested by fluorescence-activated cell sorter. Figure 2 illustrates representative results obtained by FACS in high-, intermediate-, low- and non-responders. The mean percentage of CD3⁺ (55-60%), CD14⁺ (11-13%) and CD19⁺ (14%) cells was similar in the PBMC of all responders and non-responder (64%, 9% and 16%) groups (Table 1). No significant differences were observed in the frequencies of CD3⁺, CD14⁺ and CD19⁺ cells between the subjects' groups.

DISCUSSION

The principle cellular constituents of the immune system are lymphocytes, mononuclear phagocytes and related accessory cells. Lymphocytes are the only immunocompetent cells capable of specific recognition of antigens. Response to most immunogens, with the possible exception of T cell-independent antigens, requires processing of the immunogen by (APC) [18]. Quantitative and qualitative evaluations of these cells, therefore, would provide insights into the outcome of the immune response to a nominal antigen or vaccine. Recently, we have demonstrated significantly lower HBsAg-specific B-cell frequency in HB vaccine non-responder subjects compared to responders [14]. Similar findings have also been reported for HBsAg-specific T-lymphocytes by others [13]. We and others [19, 20] showed the diminished production of both Th1 and Th2 cytokines by HBsAg-stimulated T-lymphocytes from non-responder normal individuals. These reports have prompted us to speculate on the possibility of generalized lower B and/ or T-

lymphocyte and monocyte counts in non-responder subjects. The results obtained from this study, however, did not show substantial differences between the responder and non-responder groups. All the cell types studied were similarly represented in all groups of responder individuals as well as non-responder vaccines (Table 1). Similar findings have also been reported by other investigators [8, 9]. These results do not rule out the possibility of uneven distribution of lymphocyte subpopulations between the two vaccine groups. Indeed, proportionally more CD8⁺ lymphocytes were observed in PBMC from HB non-responder vaccines [8]. These results were taken to propose presence of circulating T-lymphocytes capable of suppressing B-lymphocytes function and production of anti-HBs antibodies in the non-responder subjects. Considering the fact that the frequency of specific lymphocytes, either B or T (CD4⁺ or CD8⁺) is too low to influence the total cell count (<10⁻² - 10⁻³) [13, 14], the higher CD8⁺ T-lymphocytes reported in non-responders [8], may be polyclonal and irrelevant to the lack of antibody response in the non-responder individuals studied. Specific unresponsiveness to HBsAg may be controlled by different mechanisms, including differential expression of HLA antigens [15, 17, 21], defect in antigen specific B [7-10] or T-lymphocyte repertoire [11-13], defect in APC/T cell interactions [5, 6] and neonatal tolerance [22]. Induction of specific cytotoxic T-lymphocytes (CTL) which could recognize and destroy HBsAg specific B-lymphocytes has also been suggested [23]. In summary, specific immunological mediators, rather than generalized immune deficiency, seem to be implicated in lack of response to recombinant HB vaccine in healthy adult individuals.

Table 1. Frequency of circulating CD3, CD14 and CD19 positive cells in responders and non-responders to HB vaccine.

| Groups | CD3 ⁺ | CD19 ⁺ | CD14 ⁺ |
|-----------------------------------|-----------------------|-----------------------|--------------------|
| High-responders n = 19 | 48-79 (59 ± 20)* | 5-23 (14 ± 4) | 2-20 (12 ± 5) |
| Intermediate-responders n = 11 | 50-72 (60 ± 6) | 8-27 (14 ± 5) | 4-19 (13 ± 3) |
| Low-responders n = 9 | 45-68 (56 ± 8) | 9- 19 (14 ± 0.9) | 3-14 (11 ± 3) |
| Non-responders n = 6 | 54-84 (64 ± 10) | 4-27 (16 ± 8) | 1-16 (9 ± 5) |

*Mean ± SD.

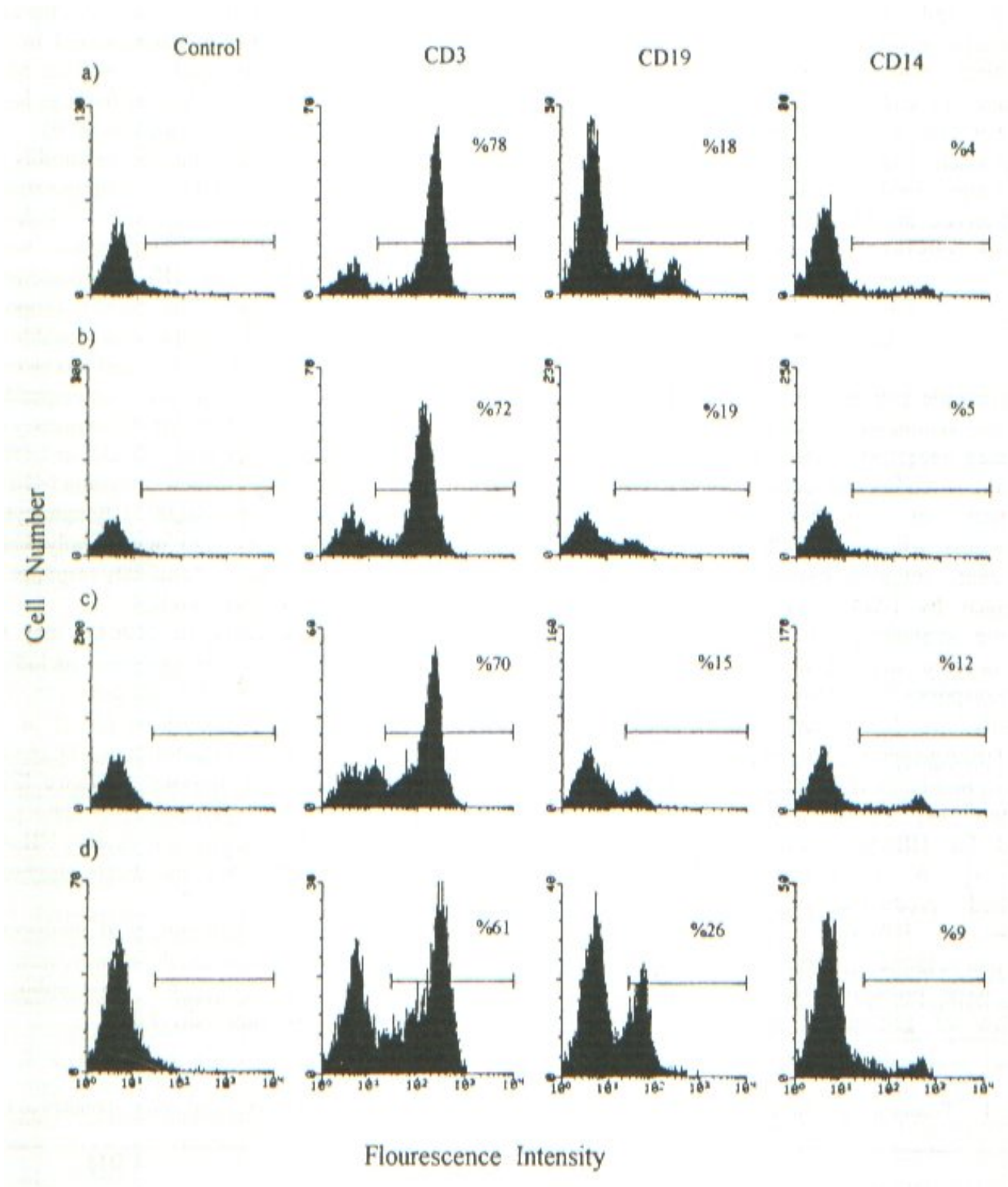


Fig. 2. FACS immunophenotypic profile of peripheral blood lymphocytes from responder and non-responder groups. a, b, c and d are representative samples from high-, intermediate-, low- and non-responders, respectively.

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REFERENCES

1. Raffaella, Z. and Guido, M. (1997) Hepatitis B vaccine: current issues. *Ann. Pharmacol.* 31: 1059-1067.
2. Amani, A. and Shokri, F. (1995) Immunogenicity of a recombinant hepatitis B vaccine in Iranian neonates: high frequency of unresponsiveness independent of the carrier state of mothers. *Iran. J. Med. Sci.* 20: 87-92.
3. Jafarzadeh, A. and Shokri, F. (2001) High rate seroconversion following administration of low doses of a recombinant hepatitis B vaccine in Iranian healthy neonates. *Vaccine* 19: 4544-4548.
4. Jenson, H.B. (2000) Non-responders to hepatitis B vaccine. *Postgrad. Med.* 107: 97-98.
5. Milich, D.R. (1987) Genetic and molecular basis for T and B cell recognition of hepatitis B viral antigens. *Immunol. Rev.* 99: 72-103.
6. Egea, E., Iglesias, A., Salazar, M., Morimoto, C., Kruskall, M.S., Awdeh, Z., Schlossman S.F., Alper C.A. and Yunis, E.J. (1991) The cellular basis for lack of antibody response to hepatitis B vaccine in humans. *J. Exp. Med.* 173: 531-538.
7. Chang, T.W., Celis, E. and Miller, R.W. (1984) *In vitro* response to HBsAg of peripheral blood lymphocytes from recipients of hepatitis B vaccine. *Hepatology* 4: 824-829.
8. Chiou, S.S., Yamauchi, K., Nakanishi, T. and Obata, H. (1988) Nature of immunological non-responsiveness to hepatitis B vaccine in healthy individuals. *Immunology* 64: 545-550.
9. Barnaba, V., Valensini, G., Levrero, M., Zaccari, C., Dyke, A.V., Falco, M., Musca, A. and Balsano, F. (1985) Immunoregulation of the *in vitro* anti-HBs antibody synthesis in chronic HBsAg carriers and in recently boosted anti-hepatitis B vaccine recipients. *Clin. Exp. Immunol.* 60: 259-266.
10. Nowicki, M.J., Tong, M.J. and Bohman, R.E. (1985) Alternations in the immune response of non-responders to the hepatitis B vaccine. *J. Infect. Dis.* 152: 1245-1248.
11. Chedid, M.G., Deulofeut, H., Yunis, D.E., Lara-Marquez, M.L., Salazar, M., Deulofeut, R., Awdeh, Z., Alper, C.A. and Yunis, J. (1997) Defect in Th1-like cells of non-responders to hepatitis B vaccine. *Hum. Immunol.* 58: 42-51.
12. Vingerhoets, J., Vanham, G., Kestens, L., Penne, G., Leroux-Roels, G. and Gigase, P. (1994) Deficient T-cell responses in non-responders to hepatitis B vaccination: Absence of Th1 cytokine production. *Immunol. Lett.* 39: 163-168.
13. Hohler, T., Meyer, C.U., Notghi, A., Stradmann-Bellinghausen, B., Schneider, P.M., Starke, R., Zepp, F., Sanger, R., Clemens, R., Meyer Zum Buschenfelde, K.H. and Rittner, C. (1998) The influence of major histocompatibility complex class II genes and T-cell repertoire on response to immunization with HBsAg. *Hum. Immunol.* 59: 212-218.
14. Shokrgozar, M.A. and Shokri, F. (2001) Enumeration of HBs antigen-specific B lymphocytes in responder and non-responder normal individuals vaccinated with recombinant HBs antigen. *Immunology* 104: 75-79.
15. Shokrgozar, M.A. and Shokri, F. (1999) HLA-associated antibody response to recombinant hepatitis B vaccine in Iranian healthy adult individuals. *Iran. J. Med. Sci.* 24: 98-103.
16. Shokri, F. and Amani, A. (1997) High rate of seroconversion following administration of a single supplementary dose of recombinant hepatitis B vaccine in Iranian healthy non-responder neonates. *Med. Microbiol. Immunol.* 185: 231-235.
17. Desombere, I., Willems, A. and Leroux-Roels, G. (1998) Response to hepatitis B vaccine: multiple HLA genes are involved. *Tissue Antigens* 51: 593-604.
18. Babbitt, B., Allen, P., Matsueda, G., Haber, E. and Unanue, E. (1985) Binding of immunogenic peptides to Ia histocompatibility molecules. *Nature* 317: 359-361.
19. Kardor, G.A., Jeddi-Tehrani, M. and Shokri, F. (2002) Diminished Th1 and Th2 cytokine production in healthy adult non-responders to recombinant hepatitis B vaccine. *Scand. J. Immunol.* 53: 1-4.
20. Larsen, C.E., Xu, J., Lee, S., Dubey, D.P., Uko, G., Yunis, E.J. and Alper, C.A. (2000) Complex cytokine response to hepatitis B surface antigen and tetanus toxoid in responders, non-responders and subjects naive to hepatitis B surface antigen. *Vaccine* 18: 3021-3030.
21. McDermott, A.B., Madrigal, J.A., Sabin, C.A., Zuckerman, J.N. and Cohen, S.B.A. (1999) The influence of host factors and immunogenetics on lymphocyte response to hepagene vaccination. *Vaccine* 17: 1329-1337.
22. Ridge, J.P., Fuchs, E.J. and Matzinger, P. (1996) Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science* 271: 1723-1726.
23. Barnaba, V., Franco, A. and Alberti, A. (1990) Selective killing of hepatitis B envelope antigen-specific B-cells by class I-restricted, exogenous antigen-specific T-lymphocytes. *Nature* 45: 258-260.