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Distinct Neopterin Excretion Patterns after Vaccination

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Summary

To compare the involvement of cellular immunity in response to vaccination we have investigated urinary neopterin levels in daily follow-ups of children after vaccination with live measles/mumps vaccine and of adults after boosting with the soluble antigen tetanus toxoid. Neopterin levels distinctly peaked 8–11 days after vaccination with measles/mumps vaccine. In contrast, after boosting with soluble antigen tetanus toxoid neopterin levels remained unaffected. Large amounts of neopterin are produced by human monocytes/macrophages on stimulation with gamma interferon. In patients neopterin concentrations reflect activation of cell mediated immunity. The data imply that distinct pathways of T cell activation are triggered in humans after immunization with live vaccine and with soluble antigen.

Introduction

The immune system comprises a complex set of components which are designed to protect host organisms against "non self" structures as, e. g., foreign pathogenes. The only immunologically specific recognition systems involve T and B lymphocytes. On these cells, specific receptors allow reaction with only one antigenic determinant. Immune responses involving these cells depend on various interactions with other cells e. g. antigen presenting cells as macrophages and accessory molecules including mediators such as lymphokines (1). During humoral immune response B-lymphocytes are activated and produce specific immunoglobulins which attack foreign structures. Activation of cell mediated immunity mainly depends on induction of the helper/suppressor T-cell subpopulations whereas cytotoxic T-cells and macrophages kill cells expressing antigenic structures. Consequences of the two alternative specific immune responses can be logistically separated to a certain extent. Of course, there are overlaps in mediators produced and consumed and in the cell types involved. Also humoral immune response depends on specific help delivered by T-lymphocytes although other possibilities for B-lymphocyte activation exist.

To protect indivduals against various pathogens which account for major health problems, vaccination is regularly in use. The organism is confronted with antigenic material similar or identical to the pathogen which induces a specific immune response. The release of specific antibodies in the circulation results from humoral immune response.

Earlier we have shown that vaccination with measles vaccine induces increase of neopterin levels in children (9). In vitro, large amounts of neopterin are produced by human macrophages on stimulation with gamma interferon (2, 3). Presence of gamma interferon characterizes activated cellular immunity. In agreement, neopterin was found to be a useful parameter in several conditions involving cellular immune activation (3, 4): for example, increasing neopterin levels allow early detection of complications in allograft recipients (5). Neopterin is a sensitive marker in viral infections including measles (6) and non-A, non-B hepatitis (7) and correlates perfectly to activity of autoaggressive disorders such as rheumatoid arthritis (8). In this study we addressed the question whether different types of vaccines behave differently to induce activation of cellular immune response in vivo. Stimulation of cellular immune response was assessed by measurement of urinary neopterin.

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Patients and Methods

During a period of 14 to 24 days urinary neopterin levels were assessed daily in 4 children (1 male, 3 females; 2.5, 3, 3 and 3.5 years old) before and after measles/mumps vaccination (M-M-Vax; Merck, Sharp & Dohme, B. V., Haarlem, Netherlands) and in 2 male adults (31 and 32 years old) after receiving tetanus toxoid booster (T Immune, Immuno AG, Vienna, Austria).

The first morning urines were used for neopterin analyses. One hundred µl of urines was diluted with 1 ml phosphate buffer (0.015 moles per liter, pH = 6.4) and injected for high pressure liquid chromatography on reversed phase C_{18} material as described previously (4, 10). Neopterin was quantified by its native fluorescence. Creatinine was measured by UV-absorption. The neopterin/creatinine ratios were used to eliminate the effect of physiologic variations in urine concentrations.

Results

In all cases the course of neopterin levels post measles/mumps vaccination showed significant peaks with distinct maxima between day 8 and day 11 (Figure 1A). Neopterin peaks were already present some days before antibody titers became usually detectable. All children remained free from any symptoms and fever. When antibodies became detectable, neopterin levels subsequently declined to baseline.

In contrast, boosting with tetanus toxoid had no effect on neopterin levels in either individual despite production of antibodies (Figure 1B).

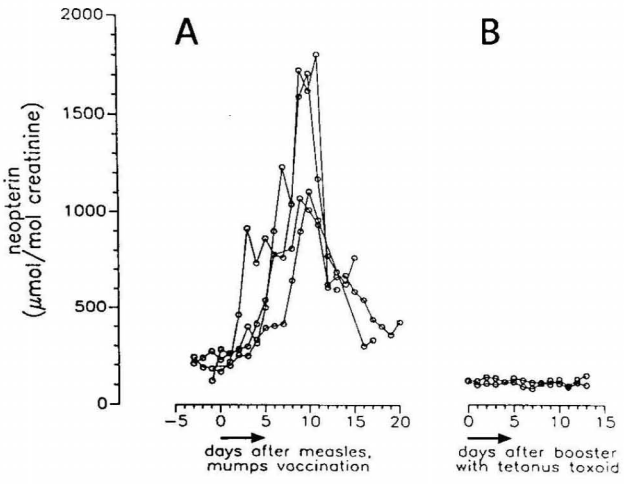

Figure 1. Urinary neopterin levels in 4 children after vaccination with live measles/mumps vaccine (A) and in 2 adults after receiving tetanus toxoid booster (B).

Discussion

Immunization with measles/mumps live vaccine and with tetanus toxoid leads to significant induction of humoral immune response which is measureable by the formation of specific antibodies. In contrast, our data demonstrate a distinct difference between both immunization procedures with respect to the involvement of cellular immune activation. Solely immunization with measles/mumps live vaccine is associated with high neopterin levels which reflects a high degree of T-cell/macrophage activation coupled with release of gamma interferon. Peaks of neopterin levels were observed several days before circulating specific antibodies can be detected. Similar courses of neopterin levels have been described during the acute cytomegalovirus infection in allograft recipients (11) and after experimental infection of rhesus monkeys with simian immunodeficiency virus (12).

In contrast, no change of neopterin levels was observed in volunteers during 15 days after boosting with tetanus toxoid. Obviously, the soluble antigen tetanus toxoid did not induce a cellular immune cascade in subjects which would have been detected by increased neopterin. Interestingly tetanus toxoid induces proliferation and release of cytokines in vitro (13) and effects on cellular immune parameters such as T-cell ratios are well established in patients after challenge with tetanus toxoid (14).

Obviously, stimulation of antibody production by vaccination with soluble antigen tetanus toxoid is not capable of inducing gamma interferon in vivo. In contrast, measles/mumps life vaccine is accompanied by release of gamma interferon similar to, e. g., allogeneic stimulation which indicates induction of cytotoxic T-cell (CTL) response. In parallel, humoral immune response is induced as it is detectable by the production of specific antibodies. Immune response, i. e., humoral and cellular immunity, involves $CD4^+$-T-helper/inducer T-cells.

Our data indicate that stimulation of an immune response by vaccination follows distinct pathways. An explanation for our result might be that different subclones of $CD4^+$ T-helper-cells are activated. As was shown recently in mice (15), a distinct $CD4^+$ T-helper/inducer cell subclone termed T_{H1} is involved mainly in cell-mediated immunity. Activation of this subclone is accompanied by release of interleukin-2 and gamma interferon but not of interleukin-4. A second $CD4^+$ T-cell subclone termed T_{H2} is engaged mainly in humoral immunity inducing B cell responses via interleukin-4 but does not release interleukin-2 or gamma interferon. If this observation holds true also in the human system, boosting with tetanus toxoid

appears to be linked solely with "help" by T_{H2}-cells which is required for antibody formation. Vaccination with live measles/mumps vaccine appears to involve activation of both, T_{H1} and T_{H2} helper/inducer T-cells as is indicated by induction of specific antibodies via T_{H2} and release of gamma interferon via T_{H1}, which was demonstrable by increase of neopterin levels.

In this study, we compared children with almost virgin immune systems with adults. The children received measles/mumps vaccine which induces a primary immune response whereas the adults were monitored after boosting with tetanus toxoid which stimulates a secondary immune response in primed individuals. The different experimental design may have contributed to the distinct neopterin patterns which were observed. Further studies are necessary to clarify this issue.

We conclude, that cell mediated immunity is involved in immunization of children with measles/mumps vaccine. Despite similar behaviour with respect to antibody formation, boosting with tetanus toxoid in adults does not involve release of gamma interferon as would be measureable by urinary neopterin concentrations. Thus, no change of neopterin excretion appears to occur in individuals exposed to vaccine solely inducing humoral immune response. Neopterin testing might serve as an easy way to detect vaccines which induce CTL-activity in humans and in primate animal models.

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References

1. Claman, H. N. (1987) J. Am. Med. Assoc. *258*, 2834–2840.
2. Huber, C., Batchelor, J. R., Fuchs, D., Hausen, A., Lang, A., Niederwieser, D., Reibnegger, G., Swetly, P., Troppmair, J. & Wachter, H. (1984) J. Exp. Med. *160*, 310–316.
3. Fuchs, D., Hausen, A., Reibnegger, G., Werner, E. R., Dierich, M. P. & Wachter, H. (1988) Immunol. Today *9*, 150–155.
4. Wachter, H., Fuchs, D., Hausen, A., Reibnegger, G. & Werner, E. R. (1989) Advances Clin. Chem. *27*, 81–141.
5. Margreiter, R., Fuchs, D., Hausen, A., Huber, C., Reibnegger, G., Spielberger, M. & Wachter, H. (1983) Transplantation *36*, 650–653.
6. Griffin, D. E., Ward, B. J., Jauregui, E., Johnson, R. T. & Vaisberg, A. (1990) J. Infect. Dis. *161*, 449–453.
7. Prior, G., Fuchs, D., Hausen, A., Judmaier, G., Reibnegger, G., Werner, E. R., Vogel, W. & Wachter, H. (1987) Lancet *ii*, 1235–1236.
8. Reibnegger, G., Egg, D., Fuchs, D., Günther, R., Hausen, A., Werner, E. R. & Wachter, H. (1986) Arthrit. Rheum. *29*, 1063–1070.
9. Reibnegger, G., Fuchs, D., Grubauer, G., Hausen, A. & Wachter, H. (1984) *in*: *Biochemical and Clinical Aspects of Pteridines* (Pfleiderer, W., Wachter, H. & Curtius, H. C., eds.), Vol. 3, Walter de Gruyter, Berlin–New York. pp. 433–447.
10. Hausen, A., Fuchs, D., König, K. & Wachter, H. (1982) J. Chromatogr. *227*, 61–70.
11. Tilg, H., Margreiter, R., Scriba, M., Marth, C., Niederwieser, D., Aulitzky, W., Spielberger, M., Wachter, H. & Huber, C. (1987) Clin. Transplantation *1*, 37–44.
12. Fendrich, C., Lüke, W., Stahl-Hennig, C., Herchenröder, O., Fuchs, D. & Wachter, H. (1989) AIDS *3*, 305–307.
13. Fuchs, D., Shearer, G. M., Boswell, R. N., Clerici, M., Reibnegger, G., Werner, E. R., Zajac, R. A. & Wachter, H. (1990) Clin. Exp. Immunol. *80*, 44–48.
14. Eibl, M. M., Mannhalter, J. W. & Zlabinger, G. (1984) N. Engl. J. Med. *310*, 198–199.
15. Mosmann, T. R. & Coffman, R. L. (1987) Two types of mouse helper T-cell clone. Implications for immune regulation. Immunol. Today *8*, 223–227.