International Immunopharmacology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Changes in antibody specificities and cytokine release after infection with lactate dehydrogenase-elevating virus

José L. Aparicio [a], Anubha Saxena [b], Jean-Paul Coutelier [b], Jacques Van Snick [c], Lilia A. Retegui [a,*]

[a] Instituto de Química y Fisicoquímica Biológicas (UBA-CONICET), Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina
[b] Unit of Experimental Medicine, Christian de Duve Institute, Université Catholique de Louvain, Brussels, Belgium
[c] Ludwig Institute for Cancer Research, Christian de Duve Institute, Université Catholique de Louvain, Brussels, Belgium

ARTICLE INFO

Article history:
Received 9 October 2012
Received in revised form 2 January 2013
Accepted 21 January 2013
Available online xxxx

Keywords:
Cryptic epitopes
Cytokine release
Anti-hGH and anti-OVA antibodies
Viral infection

ABSTRACT

Lactate dehydrogenase-elevating virus (LDV) is an apparently innocuous and persistent virus that can modify mouse immune reactions. We have shown that LDV-infected mice immunized with human growth hormone (hGH) showed a deep modification of the specificity of the anti-hGH antibodies (Ab) in CBA/Ht mice but not BALB/c animals. The aim of this work was to extend the previous observations to another mouse strain, C57BL/6, as well as to an antigen unrelated to hGH, ovalbumin (OVA), and to explore at the same time the production of various cytokines at serum and cellular levels. The amount of Ab directed to hGH or OVA native antigenic determinants versus the concentration of Ab to cryptic epitopes was evaluated by ELISA competition experiments. Results indicated that LDV infection affected Ab specificity solely in CBA/Ht mice.
In CBA/Ht the virus infection was associated with a reduction of the Ab titers to hGH native epitopes and with a decrease of IL-13 and IL-17 serum levels, but Ab to native OVA epitopes were increased with a simultaneous increase of IL-17. Accordingly, only lymph node cells from infected CBA/Ht mice immunized with OVA were found to produce INF-γ, IL-13 and IL-17.
Thus, a correlation of cytokine production with a change in Ab specificity after a viral infection was found, although this phenomenon was restricted to a given antigen and to the genetic background of immunized animals. These observations suggest that an apparent harmless virus can affect some immunological mechanisms, which could lead, for example, to inflammatory or autoimmune disorders.

1. Introduction

Lactate dehydrogenase-elevating virus (LDV) is a single-stranded positive-sense enveloped RNA nidovirus that infects mice exclusively [1]. Although infected mice develop a normal antiviral immune response, including secretion of neutralizing antibodies (Ab) and stimulation of viral-specific cytolytic T lymphocytes, the virus escapes these responses and persists lifelong in the circulation of the infected host. Importantly, subclinical LDV infection induces strong alterations of the immune microenvironment. This pro-inflammatory response is characterized by activation of innate immune cells, such as macrophages and natural killer (NK) cells [2], a burst of pro-inflammatory cytokines including type I and III interferons, interleukins 6, 12, 15 and 18 [3–5], a modulation of T helper (Th) lymphocyte differentiation towards the Th1 subtype [6] and a strong polyclonal B lymphocyte activation that results in an IgG2a-restricted hypergammaglobulinemia [7].

We have shown that infection with LDV, alone or combined with administration of adjuvants such as complete Freund's adjuvant, monophosphoryl lipid A or alum, together with immunization with human growth hormone (hGH), resulted in a profound modification of specificity in Ab to hGH [8,9]. Interestingly, this effect of LDV was dependent on the genetic background of the host and was correlated with the production of autoantibodies (autoAb) reacting with cryptic epitopes of antigens (Ag) expressed in multiple mouse organs [10].

However, since the mechanisms responsible for the LDV modulation of the Ab response are still unknown, the purpose of this work was to extend the previous observations to another mouse strain, C57BL/6, and to unrelated-hGH Ag, ovalbumin (OVA), exploring at the same time the production of various cytokines at serum and cellular levels.

2. Materials and methods

2.1. Mice

Pathogen-free BALB/c ($H2^d$), CBA/Ht ($H2^k$) and C57BL/6 ($H2^b$) mice were purchased from Harlan (Horst, The Netherlands). All animals were maintained in isolators, on standard laboratory chow, under SPF conditions until the end of the experiments, in the Unit of Experimental Medicine, de Duve Institute, Université Catholique de Louvain, Bruxelles, Belgium. The experimental protocol and animal handling

* Corresponding author at: IQUIFIB, Facultad de Farmacia y Bioquímica, Junín 956-1113 Buenos Aires — Argentina. Tel.: +54 11 4964 8289; fax: +54 11 4962 5457.
E-mail addresses: lretegui@retina.ar, liliaretegui@fibertel.com.ar (L.A. Retegui).

1567-5769/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.intimp.2013.01.016

were approved by the ethical committee of the Faculty of Medicine, Université Catholique de Louvain.

2.2. Antigens

Ovalbumin (OVA) was purchased from Sigma Chemical Co (St. Louis, MO, USA). Pituitary hGH (AFP-9755-A) was provided by the NIDDK's National Hormone and Pituitary Program and by A.F. Parlow, Harbor-UCLA Medical Center (Torrance, CA, USA).

2.3. Viral infection

Mice were inoculated intraperitoneally with 2×10^7 50% infectious doses (ID50) of LDV (Riley strain; from the American Type Culture Collection, Rockville, MD, USA) in 500 μl saline.

2.4. Adjuvant preparations and immunization protocols

Equal volumes of Complete Freund's Adjuvant (CFA) (Sigma Chemical Co., St. Louis, MO, USA) and antigen (Ag) in phosphate-buffered saline (PBS) were emulsified. Mice were inoculated subcutaneously with 200 μl of the mixture containing 25 μg of OVA or hGH; at day 15, the Ag was injected with Incomplete Freund's Adjuvant (IFA) (Sigma Chemical Co., St. Louis, MO, USA) and the animals were bled at days 30 and 60.

2.5. Cytokine assays by sandwich ELISA

Interferon (IFN-γ) was assayed by using a Ready-SET-Go kit (eBioscience, San Diego, CA) according to manufacturer's instructions, followed by the addition of 1:5000 peroxidase-conjugated avidin and Ultra-TMB-ELISA from Pierce (Rockford, IL, USA).

For IL-17 assay, microplates (Corning Incorporated, Corning, NY, USA) were coated with anti-IL-17 mAb MM178368 and captured IL-17 was revealed with biotinylated anti-IL-17 mAb MM17F3 J1127 [11] followed by a 1:5000 dilution of peroxidase-conjugated streptavidin (GE Healthcare, Buckinghamshire, UK) and Ultra-TMB-ELISA from Pierce (Rockford, IL, USA). For IL-13 assay, plates were coated with anti-IL-13 mAb and revealed with biotinylated anti-IL-13 Ab as described in [12]. IL-17A and recombinant IL-13 (R&D Systems Europe, Adington, UK) were used as standards.

2.6. Cellular cytokine production

Spleens and lymph nodes (inguinal and paraaortic) were harvested aseptically and cell suspensions were prepared after lysis of erythrocytes with ammonium chloride. Two ml of cell suspension (4×10^6 cells/ml) in Iscove's Modified Dulbecco's medium (Lonza, Walkersville, MD USA) containing 100 U penicillin/ml, 100 μg streptomycin/ml, 2 mM glutamine, 5×10–5 M 2-ME, and 10% fetal calf serum (FCS) was plated in 24-well tissue culture microplates (Costar, Corning, NY, USA). Cells were incubated alone or stimulated with 100 μg/ml of hGH or OVA according to the immunization protocol. Supernatants were collected at 72 h and stored at −20 °C until analyzed. Cytokines were assayed by ELISA as described above.

2.6. Cellular proliferation

Spleen and lymph node cells were prepared as indicated above and 100 μl of cell suspension (10^6 cells/ml) was added to 96-well flat-bottom microtiter plates (Costar, Corning, NY, USA). Cells were incubated alone or stimulated with 100 μg/ml of hGH or OVA according with the immunization protocol. After 48 h of incubation 1 μCi of [^{3}H] thymidine (PerkinElmer) was added to each well. Cells were harvested 24 h later using a semiautomatic sample harvester and radioactivity was measured in a scintillation counter. Data were processed using GraphPad Prism software (San Diego, CA, USA, http://www.graphpad.com).

2.7. ELISA to test anti-OVA and anti-hGH antibodies

ELISA microplates (Corning Incorporated, Corning, NY, USA) were coated with 100 μl of OVA or hGH at 10 μg/ml in PBS. After overnight incubation at room temperature, the plates were washed with PBS containing 0.01% Tween-20 (PBS-T) and blocked for 1 h at 37 °C with 0.01 M Tris, 0.13 M NaCl, pH 7.4 (TMS) containing 5% fetal calf serum (TMS-FCS 5%). The plates were then incubated 2 h at 37 °C with mouse serum diluted in TMS-FCS 1%, and after washing with PBS-T, the bound Ab were incubated 1 h at 37 °C with peroxidase-labeled goat anti-mouse IgG HRP (Santa Cruz Biotechnology, Ca, USA) diluted 1:5000 in TMS-FCS 1% and revealed with Ultra-TMB-ELISA from Pierce (Rockford, IL, USA).

2.8. Competition ELISA assays

ELISA microplates (Corning Incorporated, Corning, NY, USA) were coated with 100 μl of OVA or hGH (10 μg/ml) in PBS. After overnight incubation at 4 °C, the plates were washed with PBS-T and blocked for 1 h at 37 °C with 100 μl of TMS-FCS 5%. The plates were then incubated with 50 μl of mouse serum (dilution 1:5000 in TMS FCS 1%) and 50 μl of different concentrations of competitor, OVA or hGH, in TMS FCS 1%. After washing with PBS-T, the bound Ab were incubated 1 h at 37 °C with peroxidase-labeled goat anti-mouse IgG HRP (Santa Cruz Biotechnology, Ca, USA) diluted 1:5000 in TMS FCS 1%. As a substrate, ortho-phenylene-diamine-dihydrochloride (OPD, Sigma Chemical Co, St. Louis, MO, U.S.A.) with freshly added H_2O_2 was used. The reaction was stopped after 15 min by addition of 1 M H_2SO_4. The absorption was measured in an ELISA reader (Metertech Inc., Taipei, Taiwan) at 490 nm.

2.9. Statistical analysis

Statistical significance between experimental values was calculated using the Student's *t*-test.

3. Results

3.1. Effect of LDV infection on anti-hGH and anti-OVA Ab levels in BALB/c. CBA/Ht and C57BL/6 mice

Mice from each strain were separate in groups of four animals. One group of animals was LDV infected ("LDV alone"), a second was inoculated with hGH ("hGH animals"), and a third was infected with LDV and inoculated with hGH ("hGH + LDV"). Similarly, other groups of four mice were "OVA animals" or "OVA + LDV". Control mice were animals without any treatment.

The main results concerning the effect of LDV infection on Ab titers to hGH and OVA (Table 1) indicated that: i) CBA/Ht mice showed to be poor responders to hGH in comparison with the other two mouse strain since titers of Ab to hGH were lower in this mouse strain than in BALB/c or C57BL/6 animals, ii) levels of anti-OVA Ab increased after 60 days of treatment whereas Ab titers to hGH decreased with time, iii) after 60 days of treatment, LDV infection significantly increased anti-OVA Ab levels in C57BL/6 mice but slightly decreased values of Ab titers in "hGH + LDV" BALB/c animals and "OVA + LDV" CBA/Ht mice (Table 1).

3.2. Nature of the epitopes recognized by anti-hGH or anti-OVA Ab

Regarding the Ab efficacy in pathogen neutralization, one may presume that Ab to native epitopes are more beneficial than those directed

Please cite this article as: Aparicio JL, et al, Changes in antibody specificities and cytokine release after infection with lactate dehydrogenase-elevating virus, Int Immunopharmacol (2013), http://dx.doi.org/10.1016/j.intimp.2013.01.016