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

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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

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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

Table 1
Ab titers to hGH or OVA as measured by direct ELISA.

Strain	Days post-treatment	1/dilution (DO 490 nm = 1.0)			
		Treatment			
		hGH	hGH + LDV	OVA	OVA + LDV
BALB/c	30	15,000	20,000	8000	6500
	60	5500	1300	100,000	150,000
CBA/Ht	30	<1000	<1000	10,000	5500
	60	<1000	<1000	120,000	55,000
C57BL/6	30	7500	20,000	4500	9000
	60	5000	3500	1,300,000	6,000,000

ELISA microplates were coated with hGH or OVA as described in the Materials and methods section. The plates were incubated with different dilutions of sera from animals immunized and/or LDV-infected, as indicated. Bound Ab were detected with peroxidase-labeled goat anti-mouse IgG. Results are representative of two independent experiments.

against hidden epitopes — or cryptotopes. Hence, it seems appropriate to distinguish these two different specificities.

To evaluate the proportions of Ab to cryptic versus native hGH or OVA-epitopes we performed competition ELISA experiments, i.e., we measured the inhibition produced by a soluble Ag on the binding of Ab to the same insolubilized Ag. As reported previously, the Ab remaining bound to insolubilized Ag in the presence of a high concentration of the same soluble Ag should be directed against cryptic epitopes [8,9]. Thus, under our experimental conditions the curve reached the plateau when 100 μg/ml of hGH or OVA were used as competitors. For example, Ab directed to native OVA epitopes after 30 days of immunization of CBA/Ht mice should be 50%, the rest (50%) being those Ab still bound to the insolubilized Ag and so directed to its cryptic epitopes (Fig. 1A). However, LDV infection changed these figures to 80% of Ab to native epitopes and 20% of Ab to cryptotopes (Fig. 1A and Table 2), whereas no important changes of specificity were observed after 60 days of infection (Fig. 1B and Table 2). Besides, no significant change in the anti-OVA Ab specificities was observed after LDV infection in the other two mouse strains (Table 2). It is important to take into account that the apparent increased of Ab to native anti-OVA epitopes showed in "OVA + LDV" BALB/c mice after 60 days of LDV infection is a consequence of the spontaneous decrease of these Ab in the animals only immunized with the protein (Table 2).

Furthermore, virus infection somewhat enhanced the Ab population directed to cryptic hGH epitopes in BALB/c mice (30 days after treatment) and in CBA/Ht animals after 30 and 60 days of infection (Table 2).

3.3. Levels of INF-γ, IL-13 and IL-17 in serum from mice submitted to the different treatments

In order to investigate the Ag effect on the production of INF-γ, IL-13 and IL-17, we compared first the cytokine levels between "hGH" and "hGH + LDV" animals as well as "OVA" and "OVA + LDV" mice. Secondly, the cytokine serum concentration was in each case confronted with the effect of LDV alone.

Results indicated that INF-γ concentration was not detected in "hGH" animals but the cytokine levels considerably increased in serum from "hGH + LDV" C57BL/6 mice, the values being significantly higher than those of "LDV"(Table 3). INFγ was not detected or was slightly elevated in "OVA" mice and increased to some extent in "OVA + LDV" CBA/Ht animals. This cytokine level value was similar to that found in "LDV" mice (Table 3).

By contrast, serum IL-13 concentration was elevated in BALB/c and CBA/Ht "hGH" mice as well as in "OVA" CBA/Ht animals (Table 3). Cytokine amount decreased in "hGH + LDV" CBA/Ht mice to values significantly lower than in "LDV" mice, whereas an enhancement was found in "hGH + LDV" and "OVA + LDV" BALB/c animals. In those last cases IL-13 concentration did not differ from the values of "LDV" animals (Table 3).

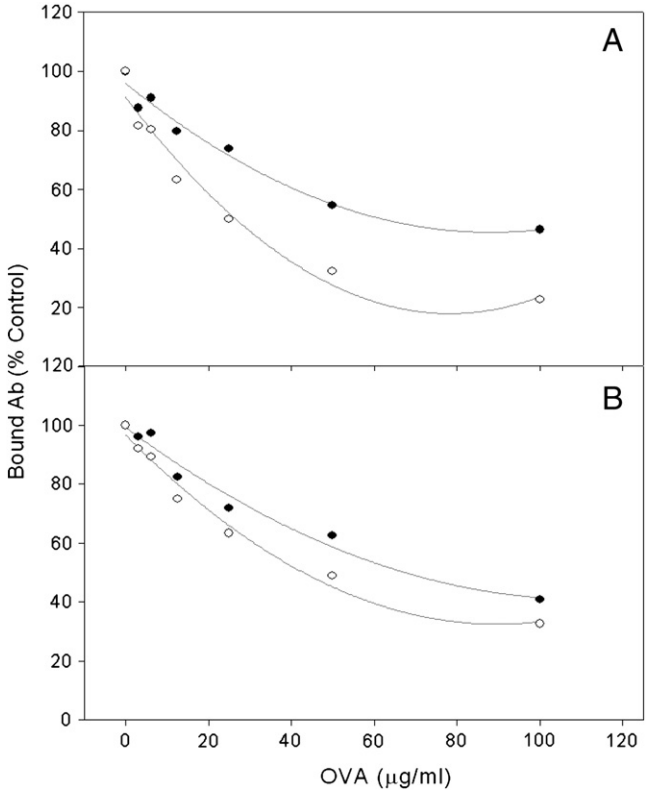

Fig. 1. Detection of Ab to native and cryptic OVA epitopes in CBA/Ht mice. ELISA microplates were coated with OVA as described in Materials and methods section. The plates were incubated with pooled sera from four mice immunized with OVA (●) or immunized and infected with LDV (○) in a dilution to obtain an optic density value of approximately 1.0 (100% of binding). Different concentrations of soluble OVA were added and bound Ab were detected with peroxidase-labeled goat anti-mouse IgG. A and B: 30 and 60 days post-infection, respectively.

Serum IL-17 was decreased in "hGH + LDV" CBA/Ht compared with "LDV" values, but increased in "OVA + LDV" BALB/c and CBA/Ht animals in comparison with "OVA" mice and even "LDV" animals (Table 3).

3.4. Effect of LDV infection on spleen and lymph node cell proliferation

Splenocytes and lymph node cells were prepared from mice submitted to the different treatments and then incubated "in vitro" with or without the addition of hGH or OVA. Results obtained with lymph node cells from CBA/Ht mice indicated that cell proliferation was higher in "hGH + LDV" mice than in "hGH" animals, and that the addition of hGH did increase the cellular growth in "hGH + LDV" animals (Fig. 2A). In a similar way, cell proliferation was more important in

Table 2
Relative percentage of Ab to hGH and OVA native epitopes in sera from mice submitted to the indicated treatments.

Mouse strain	Days post-treatment	Ab to native epitopes (%)[a]			
		Treatment			
		hGH	hGH + LDV	OVA	OVA + LDV
BALB/c	30	80	65	80	90
	60	80	85	60	80
CBA/Ht	30	25	5	50	80
	60	65	50	60	70
C57BL/6	30	80	70	95	90
	60	70	60	90	90

[a] The percent of Ab to native hGH or OVA epitopes was calculated as explained in Fig. 1 (see text).

Table 3
Determination of INF-γ, IL-13 and IL-17 in the sera of mice submitted to the indicated treatments.

Mouse strain (a)	hGH	hGH + LDV	OVA	OVA + LDV	LDV
	INF-γ (pg/ml)				
BALB/c	ND(b)	ND	ND	300 ± 190	120 ± 60
CBA/Ht	ND	ND	ND	80 ± 5^{xx}	70 ± 15
C57BL/6	ND	1830 ± 330$^{**/xx}$	110 ± 10	80 ± 1	ND
	IL-13 (pg/ml)				
BALB/c	180 ± 12	290 ± 40^{x}	ND	390 ± 30 ^{xx}	290 ± 25
CBA/Ht	440 ± 130	220 ± 80*	210 ± 80	550 ± 130	390 ± 65
C57BL/6	ND	ND	ND	ND	ND
	IL-17 (pg/ml)				
BALB/c	ND	150 ± 90	ND	240 ± 50$^{*/xx}$	95 ± 30
CBA/Ht	ND	60 ± 2$^{**/x}$	ND	200 ± 30$^{*/xx}$	90 ± 10
C57BL/6	ND	ND	ND	ND	ND

a) Cytokine levels were measured as indicated in the Materials and methods section using pools of sera from four mice submitted to each treatment. Values corresponded to 30 days post infection (triplicate sample).
b) ND: non-detected (values under 50 pg/ml).
c) Student's *t* test is indicated by *P<0.01 and **P<0.005 in comparison with values obtained from mice only infected with LDV.
d) Student's *t* test is indicated by ^{x}P<0.01 and ^{xx}P<0.005 in comparison with values obtained from mice only inoculated with hGH or OVA. The value of ND was a mean of various determinations: 45 ± 5 pg/ml.

"OVA + LDV" animals compared with "OVA" CBA/Ht mice, whereas the presence of OVA in the medium significantly augmented cell growth in "OVA + LDV" animals (Fig. 2B). Virus effect was not seen on spleen cells from the same mouse strain and neither splenocytes or lymph node cells from BALB/c or C57BL/6 animals were found reactive to hGH or OVA (data not shown).

3.5. INF-γ, IL-13 and IL-17 production by spleen and lymph node cells from CBA/Ht mice infected with LDV and/or immunized with hGH or OVA

Since CBA/Ht mice were more susceptible to LDV infection than BALB/c or C57BL/6 animals (see Fig. 1 and Tables 2 and 3), cytokine release from spleen and lymph node cells from "hGH", "hGH + LDV", "OVA" and "OVA + LDV" CBA/Ht mice were tested "in vitro" in the presence or absence of the corresponding Ag. INF-γ productions by splenocytes were similar in the various experimental conditions and a significant difference was seen upon the addition of OVA in "OVA + LDV" animals (Fig. 3). Similarly, IL-13 and IL-17 significantly augmented in "OVA + LDV" mice when the Ag was added to the cellular suspension (Fig. 3). On the other hand, only lymph node cells from "OVA + LDV" mice produced detectable amounts of the three cytokines, their concentration being independent of the addition of OVA to the culture (Fig. 3).

4. Discussion

The present work describes the characteristics of the humoral response of BALB/c, CBA/Ht and C57BL/6 mice to two different proteins inoculated with Freund's adjuvant with or without a concomitant infection with LDV. hGH was used as a control immunogen because we had found earlier that LDV shifted the specificity of the anti-hGH Ab response in CBA/Ht mice immunized with the hormone in saline, but not in BALB/c mice [8]. Here, we added a new Ag, OVA, unrelated to hGH and extended the study to a further mouse strain, C57BL/6. As INF-γ is deeply involved in the pathogenesis of LDV infection [2,13,14], this cytokine was assayed along the experimental period. IL-13 was also assayed because a depression of Th2 cytokines secretion after LDV infection has been reported [1,15]. Finally, IL-17 was also chosen because it is involved in the pathogenesis of many inflammatory and autoimmune diseases [16,17].

Results showed that BALB/c and C57BL/6 mice were better responders to hGH than were CBA/Ht mice, and that LDV infection only increased Ab concentration in C57BL/6 mice after 30 days of hGH administration (Table 1), concomitantly with a serum augmentation of INF-γ concentration value (Table 3).

Ab titers to OVA behave differently from those to hGH, since they dramatically augmented in the three mouse strains between 30 and

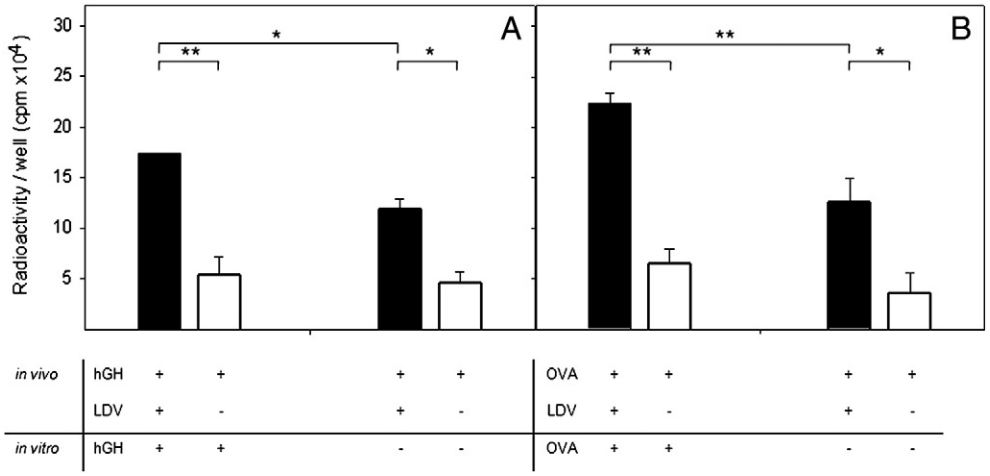

Fig. 2. Proliferation of lymph node cells from CBA/Ht mice submitted to LDV infection and/or hGH or OVA immunization. Lymph node cells were prepared as indicated in Materials and methods section and incubated alone or in the presence of 100 μg/ml of hGH or OVA in 96-well microplates. After 48 h of incubation 1 μCi/well of [^{3}H]thymidine was added to each well and cells were harvested 24 h later using a semiautomatic sample harvester. Radioactivity was measured in a scintillation counter and results are expressed as cpm/well. Student's *t* test is indicated by *P<0.01 and **P<0.005.

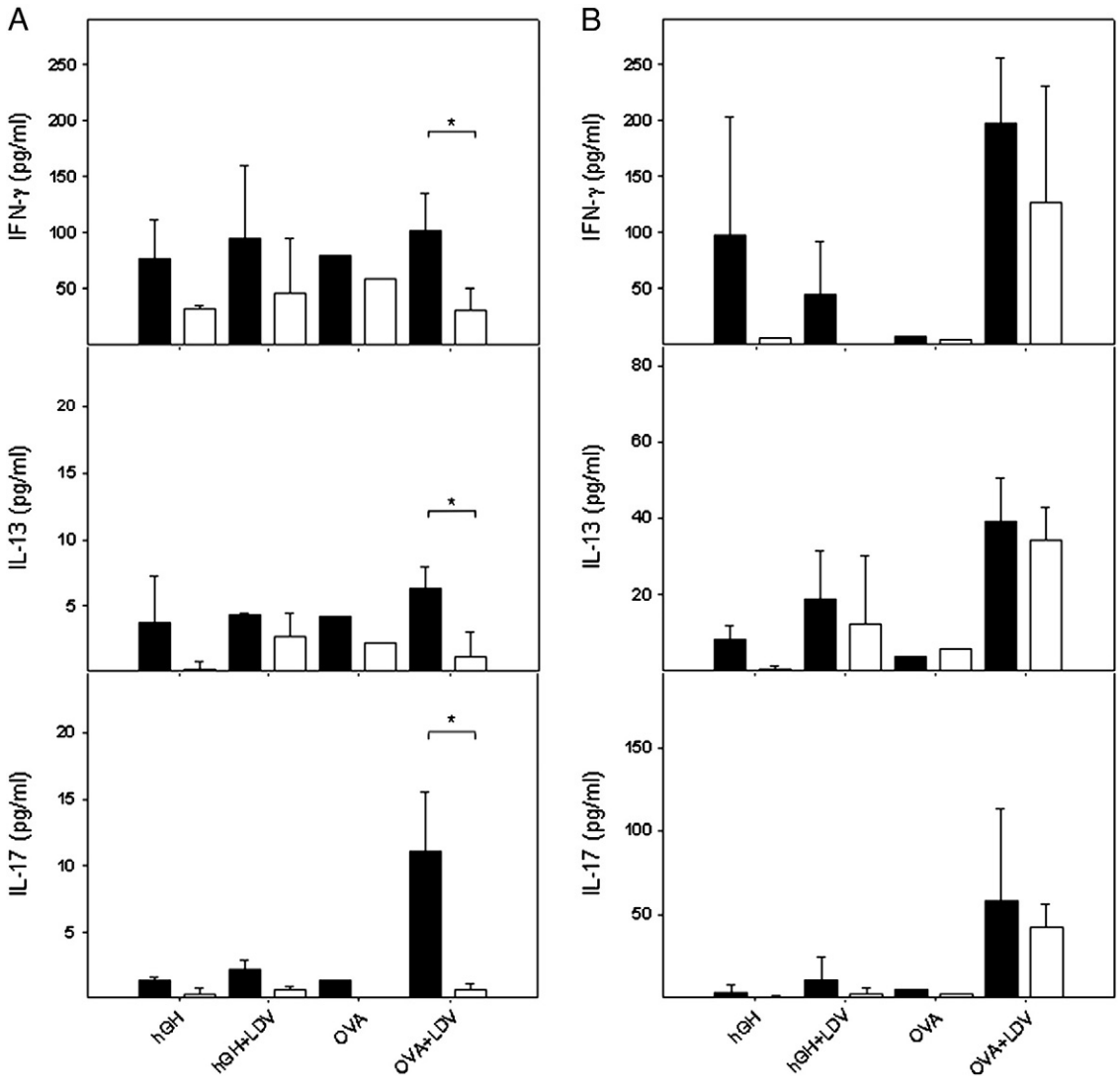

Fig. 3. Effect of LDV infection on IFN γ, IL-13 and IL-17 production by spleen (A) or lymph node (B) cells in CBA/Ht mice submitted to the indicated treatments. Spleens and lymph nodes were harvested aseptically and cell suspensions were prepared as indicated in the Materials and methods section. Cells were incubated alone (white bars) or stimulated with 100 μg/ml of hGH or OVA as indicated (black bars). Supernatants were collected at 72 h and stored at −20 °C until analyzed. Cytokines were assayed by ELISA as indicated in the Materials and methods section. Results were obtained by triplicate using pooled sera from four mice in each group and are expressed as mean ± SD. Student's *t* test is indicated by $^{*}P<0.05$.

60 days post-immunization (Table 1). In this case, LDV significantly enhanced Ab titers only in C57BL/6 mice after 60 days post-inoculation (Table 1). No correlation could be established with serum cytokine levels in this case, since neither INF-γ nor IL-13 or IL-17 were significantly increased in "OVA" C57BL/6 mice (Table 3).

Ab directed to native epitopes is considered as more effective in the neutralization of pathogens. Hence, to evaluate the efficacy of Ab elicited in each experiment, we titrated those Ab.

The lowest amount of Ab to hGH native epitopes were found in CBA/Ht mice in comparison with the two other mouse strains, and that the percentage of such Ab was even decreased by LDV infection (Table 2). Data from Table 3 show a decrease of IL-13 and IL-17 serum concentration in "hGH + LDV" CBA/Ht animals in comparison with "LDV" mice but, at the same time, a slight IL-17 increase when compared with "hGH" animals (Table 3). Those results suggest that both cytokines played a role in LDV effect at least in CBA/Ht mouse strain.

Conversely, LDV did increase the amount of Ab to native OVA epitopes in CBA/Ht mice, mainly 30 days after immunization (Fig. 1 and Table 2), whereas a slight effect was shown in BALB/c animals (Table 2). At the same time, a significant increase of serum IL-17 in "OVA + LDV" animals was observed in both mouse strains (Table 3).

Because LDV mainly replicates in a macrophage subpopulation present especially in lymph nodes, spleen, and skin [1], we tested the virus effect on the proliferation of spleen and lymph node cells. Results showed that LDV only affected lymph node cell growth from CBA/Ht mice (Fig. 2), suggesting a correlation with the strongest virus susceptibility of these animals described above (Tables 2 and 3). Negative results were obtained for the other two strains as well as for spleen cells from the three mouse strains (data not shown).

Since LDV seemed to affect preferentially CBA/Ht mice, we studied the INFγ, IL-13 and IL-17 production by spleen and node lymph cells from those animals, stimulated or not by the addition of the respective Ag "in vitro". Splenocytes from "hGH", "hGH + LDV" and "OVA" animals were found to secret INF-γ and IL-13 independently of the in vitro antigenic stimulation and/or virus infection, whereas "OVA + LDV" mice cells secreted significantly larger amounts of both cytokines in the presence of the Ag (Fig. 3). Similarly, a high IL-17 concentration was found only in cell supernatants from "OVA + LDV" animals stimulated with OVA (Fig. 3). Furthermore, INFγ, IL-13 and IL-17 production was

shown in lymph node cells from "OVA + LDV" mice, and no significant release from cells in other experimental conditions was found (Fig. 3). Surprisingly, LDV infection did not affect the characteristics of anti-hGH or anti-OVA Ab in C57BL/6 animals, which are very susceptible to infection with mouse hepatitis virus [19,20].

In most mouse strains infection with LDV does not induce overt pathology, although the virus establishes a chronic infections regardless of mouse strain, age, sex or immune status [1,18,21]. However, it has been reported that depending on the mouse strain and on the immunizing Ag, LDV may sometimes inhibit concurrent Ab responses [1], an effect observed in the present work.

Results presented herein indicated that LDV influence on Ab specificity was mainly evident in CBA/Ht mice. This observation could not be related to the "Th1" or "Th2" status of the three different mouse strains used, since CBA/Ht and C57B/6 mice has been described as Th1-like animals [22,23] and BALB/c mice as Th2-like [22].

Evidence that the C region of Ab can affect V region structure and translate into differences in affinity and/or specificity has been accumulating for some time [24,25]. It was proposed that a higher affinity or novel specificity found in an isotype switched B cell could lead to preferential binding and clonal expansion. [25]. Although at this moment no specific cytokine is known to be involved in the process described above, the strong polyclonal B lymphocyte activation produced by LDV infection [7] together with the cytokine liberation pattern observed in CBA/Ht mice – mainly IL-17 – could be responsible for the changes in specificity of Ab depicted in this paper. At the same time, since the three mouse strains are genetically different (see Materials and methods section) some non-identified gene in CBA/Ht animals could play a role in the Ab behavior. Furthermore, as a recent report suggests a role for Treg in the pathogenesis of LDV infection [26], some diversity in the Treg activity and/or Ag specificity among the three mouse strains used in this work could be responsible for the described results.

To our knowledge, this is the first time that a correlation of a raise of certain cytokines and a change of Ab specificity restricted to certain Ag after viral infection is shown. In fact, it has been reported a burst of INF-γ and IL-6, IL-12, IL-15 and IL-18 early after LDV infection, but the viral effect was transient and lasted a maximum of 48 h, depending on the cytokine considered [1]. Thus, the present results illustrate that the interaction of a "silent" virus such as LDV with the appropriate host and Ag may induce important immunological changes. It is tempting to speculate that such modifications could lead to unknown consequences and/or pathological situations depending of environmental circumstances.

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References

[1] Coutelier JP, Brinton MA. Lactate dehydrogenase-elevating virus. In: Fox James G, Davisson Muriel T, Quimby Fred W, Barthold Stephen W, Newcomer Christian E, Smith Abigail L, editors. The mouse in biomedical research, 2nd ed., vol. 2. San Diego: Elsevier/Academic Press; 2007. p. 215–34.

[2] Markine-Goriaynoff D, van der Logt JT, Truyens C, Nguyen TD, Heessen FWA, Bigaignon G, et al. IFN-γ-independent IgG2a production in mice infected with viruses and parasites. Int Immunol 2000;12:223–30.

[3] Coutelier JP, Van Broeck J, Wolf S. Interleukin-12 gene expression after viral infection in the mouse. J Virol 1995;69:1955–8.

[4] Markine-Goriaynoff D, Hulhovenb X, Cambiaso CL, Monteyne P, Briet T, Gonzalez MD, et al. Natural killer cell activation after infection with lactate dehydrogenase-elevating virus. J Gen Virol 2002;83:2709–16.

[5] Markine-Goriaynoff D, Nguyen TD, Bigaignon G, Van Snick J, Coutelier JP. Distinct requirements for IL-6 in polyclonal and specific Ig production induced by micro-organisms. Int Immunol 2001;13:1185–92.

[6] Monteyne P, Renauld JC, Van Broeck J, Dunne DW, Brombacher F, Coutelier JP. IL-4-independent regulation of in vivo IL-9 expression. J Immunol 1997;159: 2616–23.

[7] Coutelier JP, van der Logt JT, Heessen FW, Warnier G, Van Snick J. IgG2a restriction of murine antibodies elicited by viral infections. J Exp Med 1987;165:64–9.

[8] Gómez KA, Coutelier JP, Retegui LA. Changes in the specificity of antibodies in mice infected with lactate dehydrogenase-elevating virus. Scand J Immunol 1997;46:168–74.

[9] Gómez KA, Longhi SA, Marino VJ, Mathieu PA, Loureiro ME, Coutelier JP, et al. Effect of various adjuvants and a viral infection on the antibody specificity toward native or cryptic epitopes of a protein antigen. Scand J Immunol 2003;57:144–50.

[10] Gómez KA, Coutelier JP, Mathieu PA, Lustig L, Retegui LA. Autoantibodies to cryptic epitopes elicited by infection with lactate dehydrogenase- elevating virus. Scand J Immunol 2000;51:447–53.

[11] Uyttenhove C, Van Snick J. Development of an anti-IL-17A auto-vaccine that prevents experimental auto-immune encephalomyelitis. Eur J Immunol 2006;36: 2868–74.

[12] Lemaire MM, Dumoutier L, Warnier G, Uyttenhove C, Van Snick J, de Heusch M, et al. Dual TCR Expression biases lung inflammation in DO11.10 transgenic mice and promotes neutrophilia via microbiota-induced Th17 differentiation. J Immunol 2011;187:3530–7.

[13] Le-Thi-Phuong T, Thirion G, Coutelier JP. Distinct gamma interferon-production pathways in mice infected with lactate dehydrogenase-elevating virus. J Gen Virol 2007;88:3063–6.

[14] Le-Thi-Phuong T, Dumoutier L, Renauld JC, Van Snick J, Coutelier JP. Divergent roles of IFNs in the sensitization to endotoxin shock by lactate dehydrogenase-elevating virus. Int Immunol 2007;19:1303–11.

[15] Morimoto M, Yamamoto N, Hayashi T. Lactic dehydrogenase virus infection reduces the expulsion of the nematode *Nippostrongylus brasiliensis*. Parasitol Res 2003;90:153–6.

[16] Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, Gomez E, et al. Interleukins, from 1 to 37, and interferon-g: receptors, functions, and roles in diseases. J Allergy Clin Immunol 2011;127:701.

[17] Zhao L, Tang Y, You Z, Wang Q, Liang W, Han X, et al. Interleukin-17 contributes to the pathogenesis of autoimmune hepatitis through inducing hepatic interleukin-6 expression. PLoS One 2011;6:e18909.

[18] Coutelier JP, Coulie PG, Wauters P, Heremans H, van der Logt JT. In vivo polyclonal B-lymphocyte activation elicited by murine viruses. J Virol 1990;64:5383–8.

[19] Aparicio JL, Duhalde-Vega M, Loureiro ME, Retegui LA. The autoimmune response induced by mouse hepatitis virus A59 is expanded by a hepatotoxic agent. Int Immunopharmacol 2009;9:627–31.

[20] Aparicio JL, Peña C, Retegui LA. Development of autoimmune hepatitis-like disease in mice infected with mouse hepatitis virus A59 and immunized with "PADRE" adjuvant. Int Immunopharmacol 2011;11:1591–8.

[21] Ammann CG, Messer RJ, Peterson KE, Hasenkrug KJ. Lactate dehydrogenase-elevating virus induces systemic lymphocyte activation via TLR7-dependent INFα responses by plasmacitoid dendritic cells. PLoS One 2009;4:1–5.

[22] Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM. Reciprocal expression of interferon γ or interleukin 4 during the resolution or progression of murine Leishmaniasis. J Exp Med 1989;169:59–72.

[23] Detalle L, Su D, van Rooijen N, Coutelier J-P. Immunoglobulin M antiplatelet autoantibodies from mice immunized with rat platelets induce thrombocytopenia and platelet function impairment. Exp Biol Med 2010;235:1464–71.

[24] Torres M, Fernández-Fuentes N, Fiser A, Casadevall A. The immunoglobulin heavy chain constant region affects kinetic and thermodynamic parameters of antibody variable region interaction with antigen. J Biol Chem 2007;282:13917–27.

[25] Casadevall A, Janda A. Immunoglobulin isotype influences affinity and specificity. PNAS 2012;109:12272–3.

[26] Cecere TE, Todd M, LeRoith T. Regulatory T cells in arterivirus and coronavirus infections: do they protect against disease or enhance it? Viruses 2012;4:833–46.