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STIMULATION OF TH1 RESPONSE BY *HELICOBACTER PYLORI* NEUTROPHIL ACTIVATING PROTEIN DECREASES THE PROTECTIVE ROLE OF IgE AND EOSINOPHILS IN EXPERIMENTAL TRICHINELLOSIS

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Th2 responses seem to play an important role in defence against *Trichinella spiralis* (Ts). The Neutrophil Activating Protein of *Helicobacter pylori* (HP-NAP), that induces IL-12, and IL-23 expression and shifts to Th1 allergen-specific Th2 cells *in vitro* was used as an anti-Th2 agent in BALB/c mice infected with T. spiralis. The muscle larvae (ML) burden was lower ($p < 0.02$) in untreated infected animals than those infected treated with HP-NAP. In both groups there was an inverse relationship between ML burden of each animal and total IgE level (controls: r -0.617, $p = 0.0013$ and HP-NAP-treated: r -0.678, $p = 0.0001$) or eosinophil count, evaluated in the same mouse on day 42 (r -0.390, $p = 0.0592$ and r -0.803, $p = 0.0001$, respectively). Inflammatory response around the nurse cell-parasite complex was significantly higher in HP-NAP-treated infected animals than in those untreated infected, on the contrary the number of eosinophils, counted around each complex was significantly lower in the first animal group. This study provides evidence of a powerful anti-Th2 activity *in vivo* by HP-NAP and for the partial protective effect of Th2 responses in T. spiralis infection.

Trichinellosis is caused by the parasitic nematode *Trichinella*, after ingestion of raw or undercooked meat by the host. This parasite has the peculiarity of having an intracellular localization both at intestinal and muscle level where the L_1 larvae live and grow in a modified skeletal muscle fibre cell, called 'nurse cell' that induces, in encapsulating *Trichinella* species, the formation of a collagen capsule which protects the parasite from effector cells of the immune system (1). The immune response of the host at muscle level has received increasing attention in recent years (2).

IgE and eosinophils derived from the activation of Th2 cells have been considered protective against helminths for some time although, to date, *in vivo* results have provided controversial results (3-4).

T helper type 2 (Th2) cells producing cytokines such as interleukin (IL)-4, IL-5, IL-9, IL-10 and IL-13 (2, 5), are not only involved in helminth infections but also in the development of pathological

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conditions such as atopy and asthma (6-8).

A role of the above cytokines in experimental trichinellosis has been shown; in fact IL-4 plays an important role in the development of protective responses to this parasite (9). IL-4 deficiency or IL-4 blockade delayed expulsion of *Trichinella spiralis* from the small intestine (7, 10-11). Like IL-4, IL-13 is an important factor during Th2 responses, mediating mechanisms similar to those induced by IL-4, such as stimulating B cell proliferation, antibody class switching to IgE and, like IL-5, inducing eosinophilia (12-13).

After binding of both IL-4 and IL-13 to IL-4 receptor α chain (IL-4Rα) signal transducer and activator of transcription factor 6 (Stat6) are phosphorylated and activated. The transcription factor Stat6, triggered by GATA3, is necessary *in vivo* for the expulsion of *T. spiralis* and for the development of eosinophilia (14).

Other Th2-derived cytokines are important in immune responses against *T. spiralis in vivo*. As regards IL-10, knock out mice (KO) for the corresponding gene or normal mice treated with a neutralizing anti-IL-10 receptor antibody were highly susceptible to a primary *T. spiralis* infection given orally, showing significantly delayed adult worm expulsion and an increased parasite muscle burden (15). These results were not confirmed in IL-10-/- mice infected with intravenous (i.v.) injection of newborn larvae (NBL), by which the intestinal phase was by-passed (16). Furthermore, the neutralization of IL-9 by vaccination with mouse immunocromatography-purified IL-9, chemically complexed to ovalbumin or by anti-IL-9 monoclonal antibody had no significant effect on worm expulsion or muscle hypercontractility in *T. spiralis*-infected mice, although the administration of this cytokine *in vivo* enhanced jejunal muscle hypercontractility accompanying infection, and accelerated worm expulsion (17).

When IL-12 gene is over-expressed during nematode infection, the immune response is shifted from Th2 toward Th1 and worm expulsion is delayed. Moreover, the Th2 to Th1 shift abrogated the physiological responses to *T. spiralis* infection, attenuating both muscle hypercontractility and goblet cell hyperplasia (18). Accordingly, administration of exogenous IL-12 to *T. spiralis*-infected mice resulted in significantly delayed worm expulsion, increased muscle larvae (ML) burden, and remarkable decrease of Th2 cytokine secretion. The effects of exogenous IL-12 administration were largely independent of IFN-γ, as shown by obtaining the same results after IL-12 treatment of KO mice for IFN-γ.

Hence, IL-12 seems to play a significant biological role as a direct negative regulator of intestinal Th2 responses and may promote the survival of intestinal parasites *in vivo* (19).

The reciprocal antagonism of Th1 and Th2 type immune responses led us to ask whether the Th2 to Th1 shift might influence the disease outcome also in the infected muscle. To address this question, we took advantage of *Helicobacter pylori* neutrophil activating protein (HP-NAP) (20), a virulence factor derived from this bacterium, that stimulates in neutrophils high production of oxygen radicals and adhesion to endothelial cells (21). When HP-NAP was added in culture to allergen-induced human T-cell lines it shifted the cytokine profile of allergen-activated T cells from the Th2 to the Th1 cytotoxic phenotype (22). Furthermore, it was shown that this protein down-regulates Th2 responses such as eosinophilia and IgE production *in vivo* in *T. spiralis* infected mice (23), as well as in an experimental model of asthma (24).

In this study, BALB/c mice were infected with *T. spiralis*. Ten and 28 days later, infected animals were treated with intraperitoneal (i.p.) injection of PBS or HP-NAP, and the effects on the inflammatory response at muscle level, evaluated histologically semiquantitatively, were analysed at the end of the experiments on day 42 after infection, when the animals were sacrificed. ML recovered by artificial digestion were counted to evaluate the worm burden. The number of ML collected from each mouse was correlated with blood eosinophil counts, and total and *Trichinella*-specific IgE plasma levels.

MATERIALS AND METHODS

Animals

Female BALB/c mice were purchased by Harlan, Italy at 6-7 weeks of age. The animals were acclimated to the University of Pisa School of Medicine Animal Care facility for one week before the experimental infection. Food and water were continuously available throughout the experiments. All research protocols and animal care

were approved by the Ethics Committee of Pisa University in accordance with local and national regulations and approved by the University Ethics Review Committee of the Pisa University School of Medicine.

Parasite

Trichinella spiralis (code ISS003) ML were recovered from infected mice after artificial digestion in 1% pepsin (Merck KGaA, Darmstadt, Germany) in acidified water and following suspension in phosphate buffered saline (PBS), according to standard procedure. After several sedimentations ML were concentrated and counted. Experimental infection was carried out in all 56 mice using a dose of 350 ML for each mouse, administered by gavage. Then, 28 mice received intraperitoneal (i.p.) PBS on day 10 and 28 post infection (Group 1), whereas the other 28 received 10 μg of HP-NAP i.p. on day 10 and 28 post infection (Group 2).

HP-NAP protein preparation

HP-NAP was cloned, expressed and purified from *Bacillus subtilis* to avoid LPS contamination, as previously described (22). The recombinant protein was pure as judged from overloaded gels composed of different percentages of polyacrylamide. Mass spectrometry analysis, performed with a Maldi Reflex (Brucker Analytik), confirmed that the protein consisted of a single molecule of 16,875±20 Da.

T. spiralis Excretory/Secretory (TsE/S) antigen preparation

T. spiralis ML recovered by artificial digestion were cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) containing 2 mM L-glutamine and streptomycin (500 μg/ml) at 37°C in a 5% CO_2 atmosphere. After 18 h incubation, *T. spiralis* E/S antigens released in the culture surpernatant were harvested and desalted into the appropriate buffer using a PD-10 column (Amersham Pharmacia Biotech, Uppsala, Sweden). Protein concentration was estimated by absorbance at 280 nm using a Varian Cary Bio 50 spectrophotometer (Palo Alto, Ca.). Then *T. spiralis* E/S antigens were stored at -20°C until use.

Muscle parasite burden evaluation

Parasite burden evaluation was evaluated as previously described (4). Mice from Group 1 and Group 2 were sacrificed by deep ether anaesthesia at 42 days post infection (p.i.). The skinned carcass of each mouse was eviscerated and digested separately in artificial gastric juice (1% HCl and pepsin) at 37°C to calculate the number of ML for each animal.

Total IgE, specific IgE, cytokines plasma levels and blood eosinophil counts were evaluated as previously described (23).

Histology

At 42 days p.i., a sample of tongue was collected from each sacrificed animal and processed for routine histology of formalin-fixed paraffin-embedded tissues. Five μ tongue sections, at different depths, from each animal were mounted on glass slides and after routine processing, stained with haematoxylin and eosin to evaluate total inflammation, or Congo red which is suitable for eosinophil counts.

Slides were observed by a Leica Axioplan microscope and the images were acquired by a Leica video camera. Image analysis system was accomplished using an appropriate software program (Adobe® Photoshop® CS3). Through software tools, the inflammatory infiltrate around the nurse cell-parasite complex was measured in pixel calculating the difference between the whole area of nurse cell-parasite complex plus surrounding inflammation and the area delimited by the collagen capsule. The inflammatory pixel value, analysed for more than 300 larvae per experimental group on different sections, was converted in μ^2 (50 μ^2 = 78,478.8 pixel).

The number of eosinophils present in the inflammatory infiltrate around at least 50 nurse cell-parasite complexes observed per animal was counted on different tissue sections, stained with Congo red.

Statistical analysis

Results are presented as geometric means (95% confidence interval – CI) for groups of animals undergoing uniform treatment or as means ± standard deviation, depending on circumstances. Differences between groups were analyzed using the Student's two-tailed *t* test (assuming equal variances). In all cases a probability (*p*) of less than 0.05 was considered significant.

The significance of inverse correlations was calculated with student's *t* test.

RESULTS

Parasitic muscle burden evaluation

The artificial digestion of single mouse carcasses showed a relevant increase in parasite burden of Group 2 (HP-NAP infected mice), compared to Group 1 (untreated infected). In fact, while Group 1 mice had a mean of 6,517 larvae per animal, with a standard error of the mean of 762.4, HP-NAP treated animals had about a three-fold increase in parasite burden with a mean of 18,638 ± 806.5 larvae per animal ($p = 0.013$).

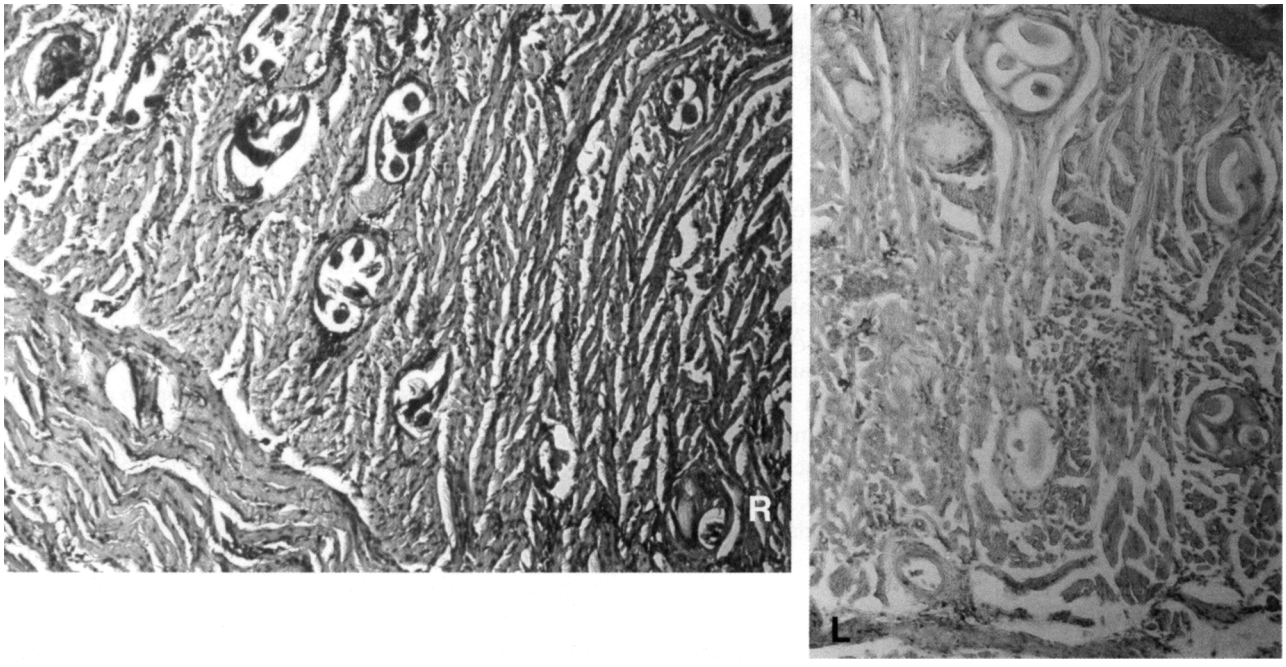

Fig. 1. *Inflammatory response in Trichinella spiralis-infected mice after HP-NAP i.p. treatment on 10th and 28th day post infection (**R**), or PBS i.p. injection (**L**) H & E stained sections of tongue muscles (original magnification 100x).*

Increased muscular pericapsular inflammation in HP-NAP treated mice

Image analysis system allowed to easily calculate the precise area of inflammation near each parasite in different sections of every mouse tongue and to receive the pixel measurement of the area. Converting pixel data in μ^2 value, a quantification of the inflammatory intensity was available. While control animals displayed a mean inflammation area of 58.655μ^2±12.059 (mean±S.D.), HP-NAP treated mice had a mean peri-capsular larval inflammation of 138.332μ2±79.991(p=0.028) Fig. 1 shows two representative pictures corresponding to histological sections characterizing the two experimental groups.

Eosinophil presence in the muscular inflammatory infiltrate of T. spiralis infected mice following HP-NAP treatment

At 42 days p.i. both HP-NAP treated and PBS-treated mice displayed the presence of eosinophils in inflammatory infiltrate surrounding the nurse cell-parasite complex. However, selective staining of eosinophils with Congo red highlighted a higher number of this cell population in the inflammatory infiltrate in the PBS-treated mice (6.44±3.377), compared to HP-NAP-treated mice (3.789±1.29 (p = 0.033).

Inverse relationship between total IgE, T. spiralis E/S-specific IgE or blood eosinophis and ML burden

To investigate whether there was any relationship between reduction of Th2 responses and the higher larvae burden found in HP-NAP treated mice, for each animal total IgE plasma level was plotted against the number of ML recovered from its carcass. In a series of 28 untreated infected animals IgE plasma levels on day 42 inversely correlated with the number of recovered larvae (r – 0.617, p = 0.0013) (Fig. 3 A). In a series of 28 HP-NAP-treated mice, the inverse relationship between total IgE levels and ML burden was even more significant (r -0.678, p = 0.0001) (Fig. 3 B).

When the same analysis was applied to *T. spiralis*

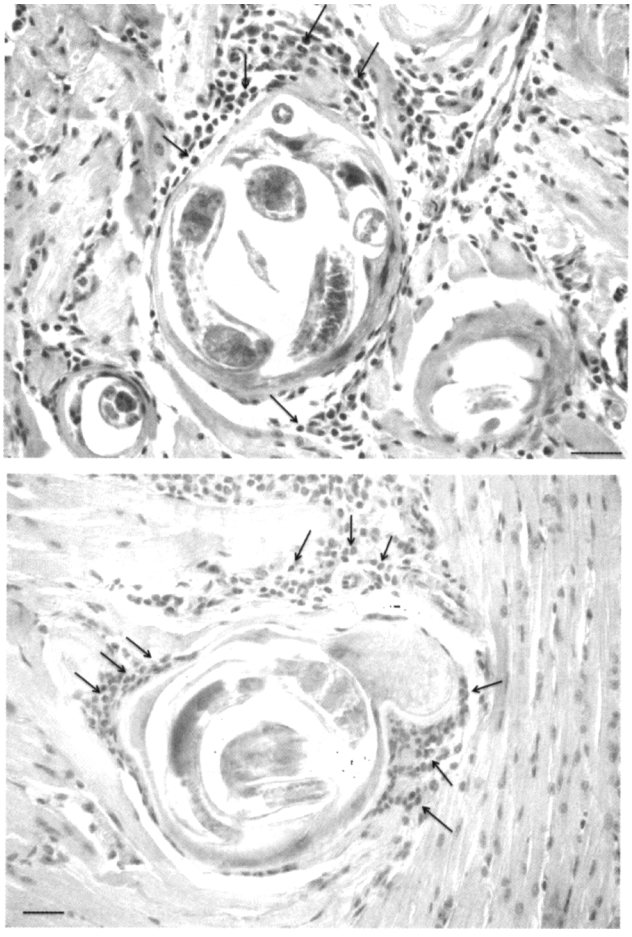

Fig. 2. *Eosinophils (arrows) surrounding the nurse cell-parasite complex in Trichinella spiralis infected mice after HP-NAP i.p. treatment on 10th and 28th day post infection B, or PBS i.p. injection (untreated controls) A. Congo Red staining of tongue muscles. Scale bar = 50* μ

E/S specific IgE, a similar inverse relationship was found in controls (r -0.529, $p < 0.01$) and more significantly in HP-NAP-treated animals (r - 0.601, $p < 0.001$) (data not shown). To further evaluate the relationship between the reduction of Th2 activity and the larvae burden, eosinophil counts in the blood on day 42 were plotted against the number of ML recovered. In Group 1 animals, there was a trend to an inverse correlation between eosinophils in the blood and the number of ML recovered (r -0.390, $p = 0.0592$) (Fig. 3 C). However, such an inverse relationship between the degree of eosinophilia and ML burden was much stronger and significant in HP-NAP treated animals (r -0.803, $p = 0.0001$) (Fig. 3 D). Moreover measures of IL-4, IL-5, IL-12plasma levels in the different conditions of infection and treatment were performed. After the first injection at day 10, plasma IL-4 and IL-5 levels were significantly lower ($p<0.0001$) in HP-NAP (198±92, 643±85, respectively) than in PBS-treated animals (605±143, 2954±247, respectively) whereas IL-12 levels were significantly higher ($p<0.0001$) in HP-NAP treated (539±124) than in non-treated (8±3) mice. Accordingly, after the second injection of HP-NAP on day 28, both IL-4 and IL-5 levels were lower in HP-NAP treated mice, whereas IL-12 levels were higher in HP-NAP treated mice (data not shown).

DISCUSSION

The experimental trichinellosis model offers the great advantage for immunomodulation *in vivo* studies to evaluate the possible effects of molecules in studies on the worm burden at a definitive time point of infection. In our study, worm burden increases significantly about three times in HP-NAP-treated infected animals compared to PBS-treated mice. These results, in agreement with those obtained by Helmby and Grencis (19), indicate that when the Th1 response is reinforced, the host is less able to control infection. Considering that the first dose of HP-NAP was administered in our study to mice on the 10th day post infection, when the intestinal phase is not yet terminated in BALB/c mice (25), we hypothesize an alteration of the intestinal immune response due to the Th1 adjuvant effect of HP-NAP. Preliminary data show that when HP-NAP is given the same day as oral infection a delay of adult worm expulsion from the intestine is observed. We previously showed that double injection of HP-NAP between the end of intestinal and early muscular phase of experimental trichinellosis in BALB/c mice (10th and 28th days post infection) was able to down-regulate Th2 response (23). High-responder mice, indeed, were found to produce higher levels of anti-*T. spiralis* IgE, while showing lower worm burden (3). Our study confirms such a notion, since a significant inverse relationship was found between

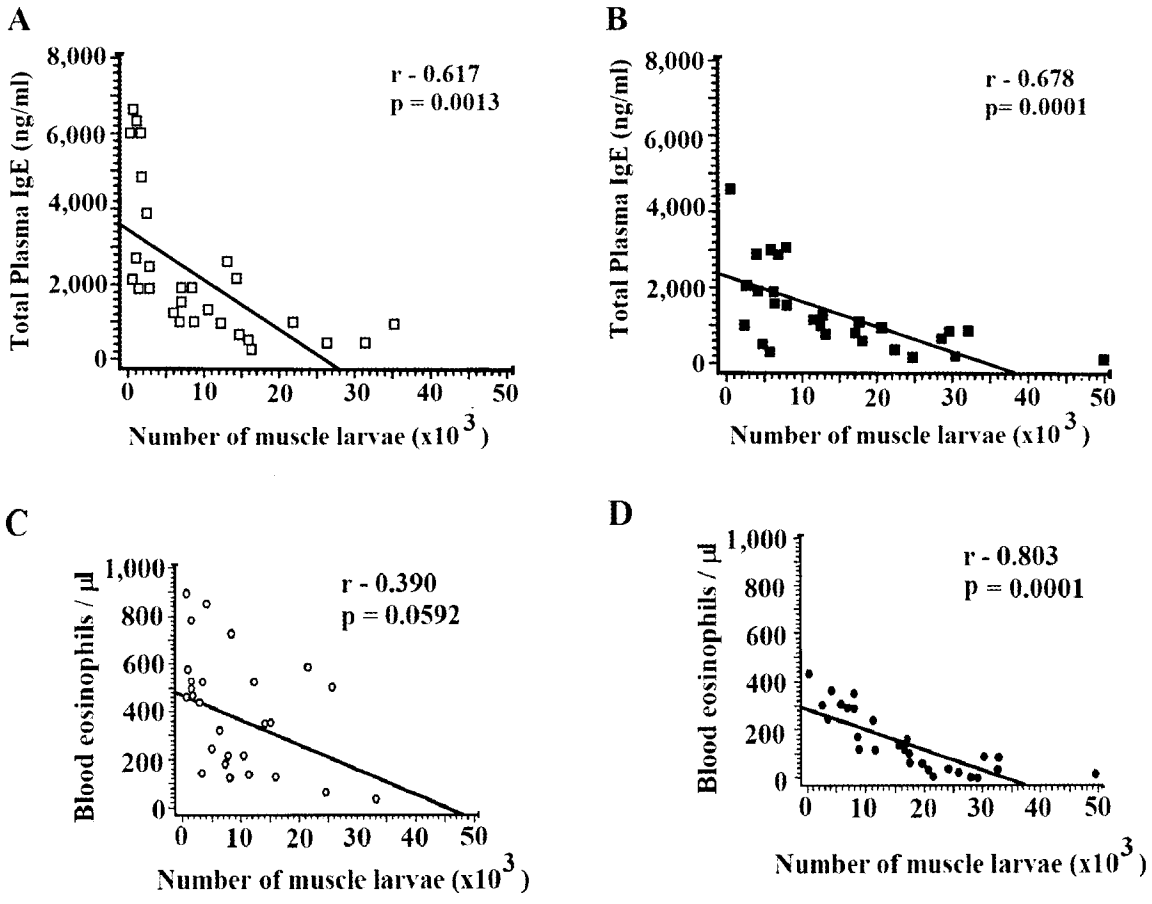

Fig. 3. *Inverse relationship between total IgE/blood eosinophilia and muscle larvae burden (ML). For each animal (28 controls and 28 HP-NAP-treated animals) both total IgE plasma level and eosinophil counts in the blood on day 42 were plotted against the number of ML recovered from its carcass after artificial digestion. A) In control animals (open squares) IgE plasma levels on day 42 inversely correlated with the number of recovered larvae (r – 0.617, p = 0.0013). B) In HP-NAP-treated mice (closed squares), the inverse relationship between total IgE levels and ML burden was even more significant (r -0.678, p = 0.0001). C) In control animals, there was a trend to an inverse correlation between eosinophils in the blood and the number of ML recovered (r -0.390, p = 0.0592), whereas (D) in HP-NAP treated animals the inverse relationship between eosinophil counts and ML burden was much stronger and significant (r -0.803, p = 0.0001).*

total IgE or *T. spiralis* E/S-specific IgE plasma levels and the number of ML recovered from carcasses in both controls and HP-NAP treated mice.

Thus, when blood eosinophil counts on day 42 in controls were plotted against the numbers of ML recovered, a trend to an inverse relationship was found, but it was not statistically significant. In contrast, in animals undergoing the anti-Th2 activity of HP-NAP, such an inverse relationship was much more convincing and significant, indicating a protective effect of Th2 response versus Trichinella.

Studies on *T. spiralis* immune response (at muscle level) are mostly based on the immunomodulation of infection by treatment with cytokine-specific monoclonal antibody (26) or genetic modification in transgenic animals (27). Using KO mice (28) or IgE KO mice (29), it was demonstrated that Th2 responses resulted beneficial for the host infected by T. spiralis. The intracellular localization of larvae in the muscles makes trichinellosis a peculiar model of helminth infection. Fabre et al. have, conversely, described a protective role of eosinophils even for the parasite (30).

Results obtained from several experimental

studies seem to diverge from each other, according to the mouse model used (4). In our mouse model the modulation of the immunological response during *Trichinella* infection is the result of HP-NAP administration: this molecule of bacterial origin is able to interact with different cells of the innate immune system (21-22, 31-32) directing differentiation of T helper cells toward a Th1 activation *in vitro* (22) and *in vivo* (23-24).

Focusing on the muscle phase of the parasite, we found evidence of histological changes such as an increase of the inflammatory infiltrate and a decrease of eosinophils surrounding the nurse cell-parasite complex in HP-NAP-treated compared to PBS-treated mice. High intensity in the inflammation infiltrate in HP-NAP-treated mice is still present 42 days post oral infection in our experimental conditions, probably owing to chemoattractant activity of HP-NAP (21). One can speculate that the higher inflammation observed in HP-NAP-treated animals might be due to the higher number of ML present in the muscle tissue, but it has to be observed that our image analysis system permitted to appreciate the inflammatory infiltrate around each NC-parasite complex, which is independent on the parasite burden.

Such an increase in inflammatory cell infiltration in Group 2 was similarly observed in IL-10 -/- infected mice, by i.v. injection of NBL (by-passing the enteral phase), during the early phase of infection (20 days post muscle infection) when the lack of the cytokine facilitates the tissue accumulation of inflammatory cells (18). However, at 50 days no difference was observed between KO and wild type mice, suggesting that at this time IL-10 does not regulate tissue inflammation (16). Despite the relevant differences as regards inflammatory infiltrate around the parasite, the worm burden at muscle level was similar in the two animal groups, neither infectivity of muscle larvae, recovered from infected animals resulted different, indicating that the exaggerated inflammatory response does not give advantages to the host. The Th1 redirection, induced by HP-NAP, leads to the down-regulation of the cytokine production by Th2 effector cells (23). Th2 cells are not only the source of IL-4 and IL-5 but also of IL-10 in *Trichinella* model (16). We can speculate that also in our experimental conditions the IL-10 level reduction resembles that which drastically occurs in IL-10 -/- infected mice (16).

On the other hand, HP-NAP is able to stimulate extravasation of inflammatory cells, not only recruiting leukocytes from the vascular lumen, and to stimulate leukocytes to release mediators which favour the maintenance of the inflammatory process (21). Furthermore, it has been shown that the protein increases the half life of monocytes and neutrophils (22). According to Beiting et al. (16), macrophages are the predominant cells involved in the inflammatory infiltrate surrounding the nurse cell-parasite complex, whereas lymphocytes are largely CD4+, with fewer CD8+ T cells and rare B cells.

The number of eosinophils, evaluated in the pericapsular inflammatory infiltrate surrounding the NC-parasite complex, resulted lower in HP-NAP-treated mice compared to PBS-treated mice. This means that the decrease of blood eosinophils observed in HP-NAP-treated mice is not due to a tissue accumulation of eosinophils, but rather to a reduced IL-5 production, as shown previously when the levels of this cytokine resulted significantly lower after each injection of HP-NAP (23).

In the presence of the anti-Th2 activity of HP-NAP, ML burden was significantly higher. These data also provide indirect evidence for the partial protective effect of Th2 responses raised by the host in *T. spiralis* infection. Furthermore, HP-NAP was shown to modulate *in vivo* myositis which represents a hallmark of trichinellosis. Taken together, these data suggest that in terms of IgE antibodies and eosinophilia, the weaker was the *T. spiralis*-induced Th2 response, the higher being the chance for the infected animals to let parasitic larvae survive in their muscles.

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M.d.B., G.D.P, M.M.D.E., A.A. are applicants of EU Patent 05425666.4 for HP-NAP as a potential therapeutic agent in cancer, asthma, allergic and infectious diseases. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Unfortunately, Prof. Gianfranco Del Prete passed away last year.

REFERENCES

1. Despommier DD. How does *Trichinella spiralis* make itself at home. Parasitol Today 1998; 14:318-23.
2. Fabre MV, Beiting DP, Bliss SK, Appleton JA. Immunity to *Trichinella spiralis* muscle infection. Vet Parasitol 2009; 159:245-48.
3. Watanabe N, Bruschi F, Korenaga M. IgE: a question of protective immunity in *Trichinella spiralis* infection. Trends Parasitol 2005; 21:175-78.
4. Bruschi F. Untangling the role of eosinophils in trichinellosis: a never-ending story. Trends Parasitol 2010; 26:60-61.
5. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989; 7:145-73.
6. Del Prete G, De Carli M, D'Elios MM, et al. Allergen exposure induces the activation of allergen-specific Th2 cells in the airway mucosa of patients with allergic respiratory disorders. Eur J Immunol 1993; 23:1445-49.
7. Else KJ, Finkelman FD, Maliszewski CR, Grencis RK. Cytokine-mediated regulation of chronic intestinal helminth infection. J Exp Med 1994; 179: 347-51.
8. Urban JF Jr, Noben-Trauth N, Donaldson DD, Madden KB, Morris SC, Collins M, Finkelman FD. IL-13, IL-4Ralpha, and Stat6 are required for the expulsion of the gastrointestinal nematode parasite *Nippostrongylus brasiliensis*. Immunity 1998; 8: 255-64.
9. Scales HE, Ierna MX, Lawrence CE. The role of IL-4, IL-13 and IL-4Ralpha in the development of protective and pathological responses to Trichinella spiralis. Parasite Immunol 2007; 29:81-91.
10. Bancroft AJ, McKenzie AN, Grencis RK. A critical role for IL-13 in resistance to intestinal nematode infection. J Immunol 1998; 160:3453-61.
11. Lawrence CE, Paterson JC, Higgins LM, MacDonald TT, Kennedy MW, Garside P. IL-4-regulated enteropathy in an intestinal nematode infection. Eur J Immunol 1998; 28:2672-84.
12. Emson CL, Bell SE, Jones A, Wisden W, McKenzie AN. Interleukin (IL)-4-independent induction of immunoglobulin (Ig) E, and perturbation of T cell development in transgenic mice expressing IL-13. J Exp Med 1998; 188:399–404.
13. Gessner A, Mohrs K, Mohrs M. Mast cells, basophils, and eosinophils acquire constitutive IL-4 and IL-13 transcripts during lineage differentiation that are sufficient for rapid cytokine production. J Immunol 2005; 174:1063-72.
14. Khan WI, Blennerhasset P, Ma C, Matthaei KI, Collins SM. Stat6 dependent goblet cell hyperplasia during intestinal nematode infection. Parasite Immunol 2001; 23:39-42.
15. Helmby H, Grencis RK. Contrasting roles for IL-10 in protective immunity to different life cycle stages of intestinal nematode parasites. Eur J Immunol 2003; 33:2382-90.
16. Beiting DP, Gagliardo LF, Hesse M, Bliss SK, Meskill D, Appleton JA. Coordinated control of immunity to muscle stage *Trichinella spiralis* by IL-10, regulatory T cells, and TGF-beta. J Immunol 2007; 178:1039-47.
17. Khan WI, Richard M, Akiho H, Blennerhasset PA, Humphreys NE, Grencis RK, Van Snick J, Collins SM. Modulation of intestinal muscle contraction by interleukin-9 (IL-9) or IL-9 neutralization: correlation with worm expulsion in murine nematode infections. Infect Immun 2003; 71:2430-38.
18. Khan WI, Blennerhassett PA, Deng Y, Gauldie J, Vallance BA, Collins SM. IL-12 gene transfer alters gut physiology and host immunity in nematode-infected mice. Am J Physiol Gastrointest Liver

Physiol 2001; 281:102-10.

19. Helmby H, Grencis RK. IFN-gamma-independent effects of IL-12 during intestinal nematode infection. J. Immunol 2003; 171:3691-96.
20. Dundon WG, Nishioka H, Polenghi A, Papinutto E, Zanotti G, Montemurro P, Del GG, Rappuoli R, Montecucco C. The neutrophil-activating protein of *Helicobacter pylori*. Int J Med Microbiol 2002; 291: 545-50.
21. D'Elios MM, Montecucco C, de Bernard M. VacA and HP-NAP, Ying and Yang of Helicobacter pylori-associated gastric inflammation. Clin Chim Acta. 2007; 381:32-38.
22. Amedei A, Cappon A, Codolo G, Cabrelle A, Polenghi A, Benagiano M, Tasca E, Azzurri A, D'Elios MM, Del Prete G, de Bernard M. The Neutrophil Activating Protein of *Helicobacter pylori* promotes T helper type 1 immune responses. J Clin Invest 2006; 116:1092-101.
23. Del Prete G, Chiumiento L, Amedei A, Piazza M, D'Elios MM, Codolo G, de Bernard M, Masetti M, Bruschi F. Immunosuppression of TH2 responses in *Trichinella spiralis* infection by *Helicobacter pylori* neutrophil-activating protein. J Allergy Clin Immunol 2008; 122:908-13.
24. Codolo G, Mazzi P, Amedei A, Del Prete G, Berton G, D'Elios MM, de Bernard M. The neutrophil-activating protein of *Helicobacter pylori* down-modulates Th2 inflammation in ovalbumin-induced allergic asthma. Cell Microbiol 2008; 10:2355-63.
25. Dea-Ayuela MA, Rama-Iñiguez S, Bolas-Fernández F. Vaccination of mice against intestinal *Trichinella spiralis* infections by oral administration of antigens microencapsulated in methacrilic acid copolymers. Vaccine 2006; 24:2772-80.
26. Herndon FJ, Kayes SG. Depletion of eosinophils by anti-IL-5 monoclonal antibody treatment of mice infected with *Trichinella spiralis* does not alter parasite burden or immunologic resistance to reinfection. J Immunol 1992; 149:3642-47.
27. Hokibara S, Takamoto M, Tominaga A, Takatsu K, Sugane K. Marked eosinophilia in interleukin-5 transgenic mice fails to prevent *Trichinella spiralis* infection. J Parasitol 1997; 83:1186-89.
28. Gurish MF, Humbles A, Tao H, Finkelstein S, Boyce JA, Gerard C, Friend DS, Austen KF. CCR3 is required for tissue eosinophilia and larval cytotoxicity after infection with *Trichinella spiralis*. J Immunol 2002; 168:5730-36.
29. Gurish MF, Bryce PJ, Tao H, Kisselgof AB, Thornton EM, Miller HR, Friend DS, Oettgen HC. IgE enhances parasite clearance and regulates mast cell responses in mice infected with *Trichinella spiralis*. J Immunol 2004; 172: 1139-45.
30. Fabre V, Beiting DP, Bliss SK, Gebreselassie NG, Gagliardo LF, Lee NA, Lee JJ, Appleton JA. Eosinophil Deficiency Compromises Parasite Survival in Chronic Nematode Infection. J Immunol 2009; 182:1577-83.
31. Satin B, Del Giudice G, Della Bianca V, et al. The neutrophil-activating protein (HP-NAP) of *Helicobacter pylori* is a protective antigen and a major virulence factor. J Exp Med 2000; 191:1467-76.
32. Cappon A, Babolin C, Segat D, Cancian L, Amedei A, Calzetti F, Cassatella MA, D'Elios MM, de Bernard M. *Helicobacter pylori*-derived neutrophil-activating protein increases the lifespan of monocytes and neutrophils. Cell Microbiol 2010; 12:754-64.