Cytokines / NO Synthase 2” system in cystic echinococcosis: Immune mechanisms and biotherapeutic implications

Manel Amri[1] **&** Chafia Touil-Boukoffa [2]

1: Cytokines and NOSynthases team, Laboratory of Cellular and Molecular Biology (LBCM), Faculty of Biological Sciences (FSB), University of sciences and technology Houari Boumediene (USTHB), Algiers, Algeria
2: Algerian Academy of Science and Technology (AAST), Algiers Algeria

COLLABORATIONS

- National university hospitals
- International research centers and universities

TEAM THEMES

They are structured around a vision of:

Basic research

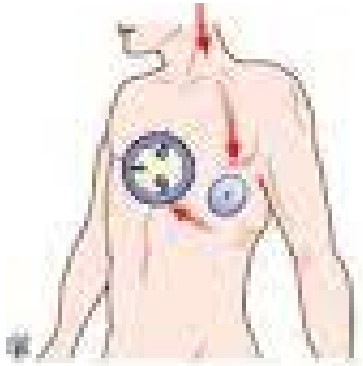
Applied research approach

SCIENTIFIC APPROACH

Part 1

Evaluate the implication of Cytokines / NO Synthases in immun responses modulation during CE

In vivo **(sera, immune cells) during human CE**



In vivo **in an experimental model of murin echinococcosis**

PUBLICATIONS

Biochimie
Volume 80, Issues 8–9, August–September 1998, Pages 739-744

Production of nitric oxide (NO) in human hydatidosis: Relationship between nitrite production and interferon-γ levels

Chafia Touil-Boukoffa [a], Brigitte Bauvois [b], Josiane Sancéau [b], Boussad Hamrioui [a], Juana Wietzerbin [a,b]

Comparative study of nitric oxide (NO) production during human hydatidosis: relationship with cystic fluid fertility

- Razika Zeghir-Bouteldja[1],
- Manel Amri[1],
- Samia Bouaziz[1],
- Dalila Mezioug[1] &
- Chafia Touil-Boukoffa[1]

Parasitology Research volume 112, pages 649–654 (2013) Cite this article

Journal of Interferon & Cytokine Research, VOL. 17, NO. 4 |

normal

Relationship Among Circulating Interferon, Tumor Necrosis Factor-α, and Interleukin-6 and Serologic Reaction Against Parasitic Antigen in Human Hydatidosis

CHAFIA TOUIL-BOUKOFFA, JOSIANE SANCËAU, BOUCHENTOUF TAYEBI, and JUANA WIETZERBIN

Acta Tropica
Volume 203, March 2020, 105306

Potential role of NF-κB pathway in the immuno-inflammatory responses during human cystic echinococcosis

Sarah Tilioua [a], Dalila Mezioug [a], Zine-Charaf Amir-Tidadini [b], Yacine-Miloud Medjdoub [c], Chafia Touil-Boukoffa [a]

Alterations in interferon-gamma and nitric oxide levels in human echinococcosis.

Ait Aissa S[1], Amri M, Bouteldja R, Wietzerbin J, Touil-Boukoffa C

Author information

Cellular and Molecular Biology (Noisy-le-Grand, France), 15 May 2006, 52(1):65-70
PMID: 16914097

112 Eur. Cytokine Netw. Vol. 23 n° 3, *July-August-September 2012*, 112-9

RESEARCH ARTICLE

Interleukin-17A correlates with interleukin-6 production in human cystic echinococcosis: a possible involvement of IL-17A in immunoprotection against *Echinococcus granulosus* infection

Dalila Mezioug, Chafia Touil-Boukoffa

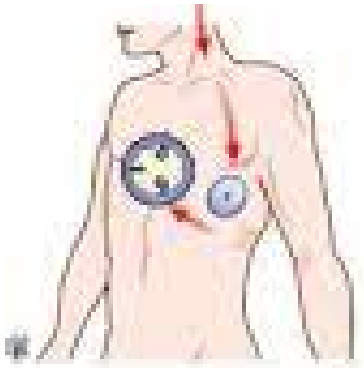
Article available at http://www.parasite-journal.org or http://dx.doi.org/10.1051/parasite/2009161057

ÉTUDE DU PROFIL CYTOKINIQUE DE PATIENTS ATTEINTS D'HYDATIDOSE : UNE POSSIBLE APPLICATION EN MATIÈRE D'IMMUNOSURVEILLANCE

MEZIOUG D.* & TOUIL-BOUKOFFA C.*

Vuitton, Gottstein, Siracusano, Rigano, Zhang, Mc Mannus, Diaz, Da silva, Romig, Rogan, Bruneti, Lightowlers, Ferreira, Siles Lucas, ...)

RESULTS 1

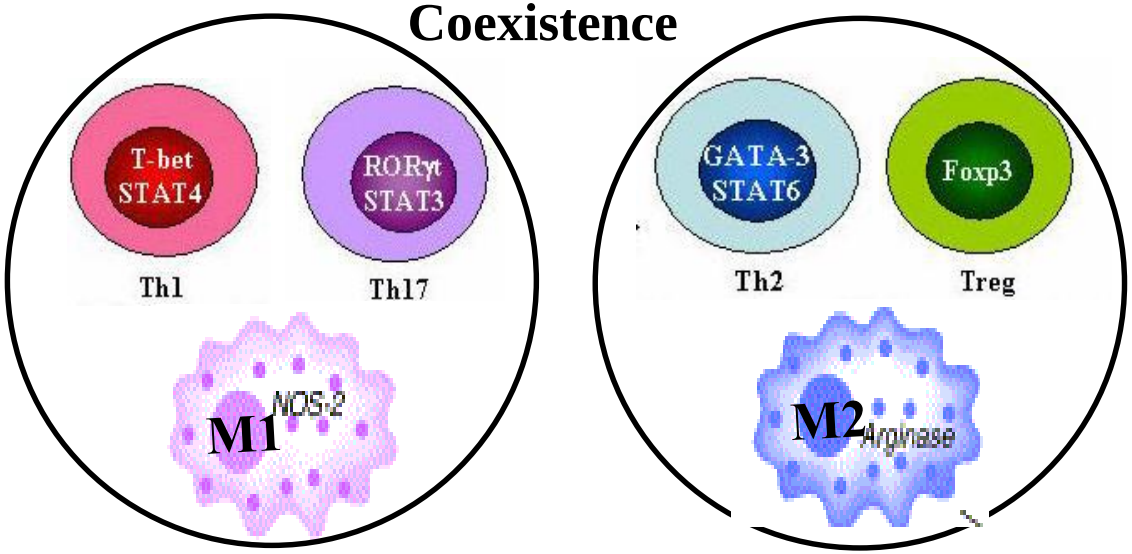


Patients with CE (before surgery)

In vivo
(sera, immune cells)

Coexistence

Inflammation



Regulation of inflammation

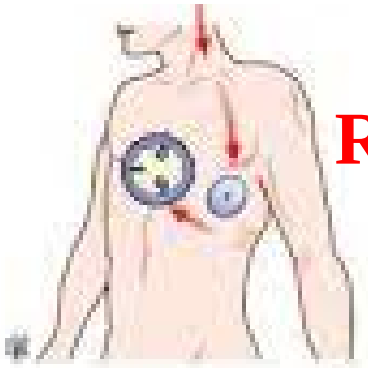
CONCLUSION 1

This implies the existence of an immun equilibrium

This may explain the lack of effective parasite rejection, even in the presence of specific immune responses

Suggests the existence of complex and evolving escape and survival strategies induced by the parasite

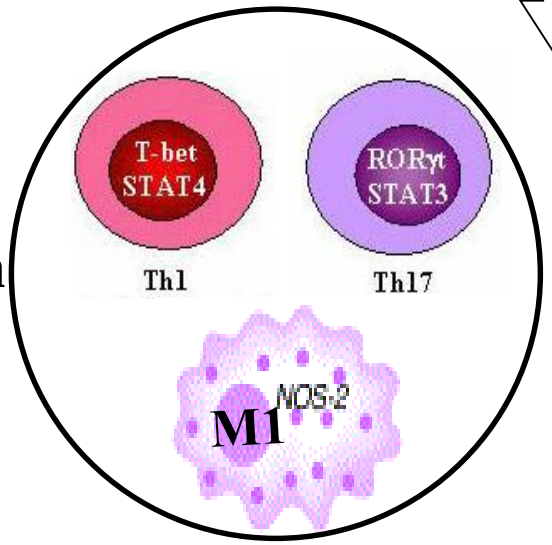
RESULTS 2

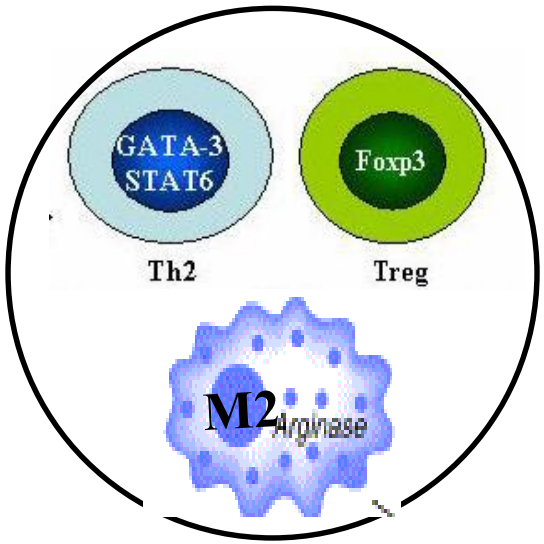


Relapsing patients with CE (before surgery)

In vivo
(sera)

Inflammation





Regulation of inflammation

Good prognosis

Bad prognosis

CONCLUSION 2

These results highlight the potential of these cytokines, notably IFN-γ and IL-4, as biomarkers of interest in the clinical monitoring and prognosis of patients with CE

These data confirm the existence of the survival strategies induced by the parasite

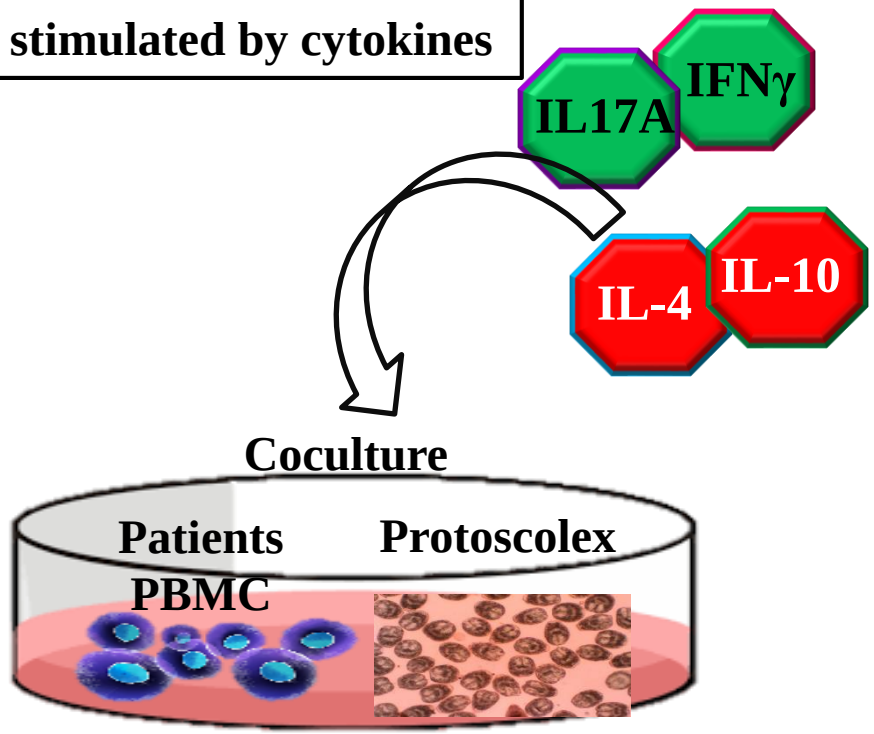
SCIENTIFIC APPROACH

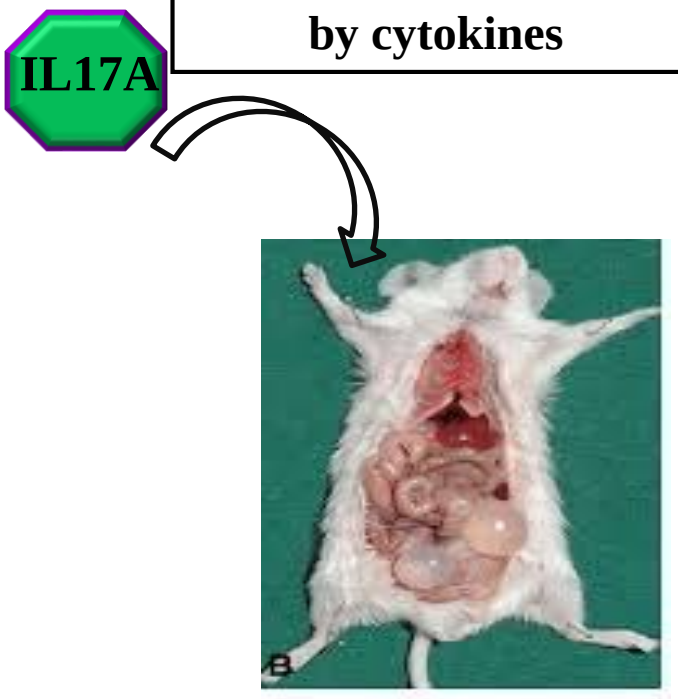
Part 2

Identify the molecular mechanisms involved in parasite survival or susceptibility

Ex vivo **on Patients PBMCs-PSC cocultures stimulated by cytokines**

In vivo **in an experimental model of echinococcosis treated by cytokines**





TEAM PUBLICATIONS

JOURNAL OF INTERFERON & CYTOKINE RESEARCH 27:781–787 (2007)
© Mary Ann Liebert, Inc.
DOI: 10.1089/jir.2007.0003

In Vitro Antihydatic Action of IFN-γ Is Dependent on the Nitric Oxide Pathway

MANEL AMRI, SALIHA AIT AISSA, HOUDA BELGUENDOUZ, DALILA MEZIOUG, and CHAFIA TOUIL-BOUKOFFA

Eur. Cytokine Netw., Vol. 20 n° 2, June 2009, 63-8

RESEARCH ARTICLE

Involvement of IL-10 and IL-4 in evasion strategies of *Echinococcus granulosus* to host immune response

Manel Amri, Dalila Mezioug, Chafia Touil-Boukoffa

Acta Tropica 181 (2018) 6–10

Contents lists available at ScienceDirect

Acta Tropica

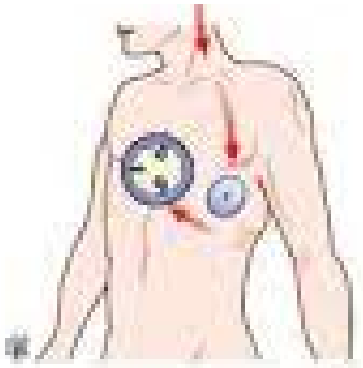
journal homepage: www.elsevier.com/locate/actatropica

In vivo treatment with IL-17A attenuates hydatid cyst growth and liver fibrogenesis in an experimental model of echinococcosis

Moussa Labsi[a], Imene Soufli[a], Lila Khelifi[a], Zine-Charaf Amir[b], Chafia Touil-Boukoffa[a,*]

[a] Laboratory of Cellular and Molecular Biology, Department of Biology, University of Sciences and Technology Houari Boumediene. Algiers-Algeria
[b] Department of Anatomy and Pathological Cytology, University Hospital Center Mustapha Pacha, Algiers-Algeria

RESULTS 3



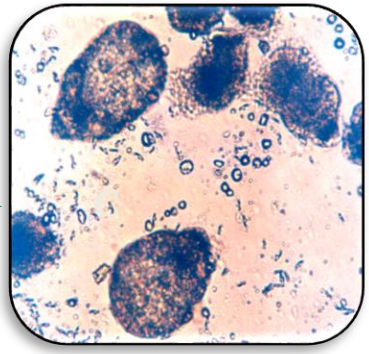
Patients with CE (before surgery)

Scolicidal

+ IFN-γ

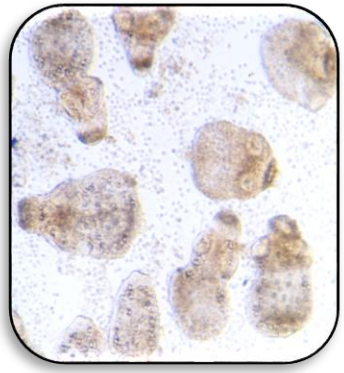
or IL-17A

After 20h



Ex vivo
(Patients PBMC- PSC cocultures)

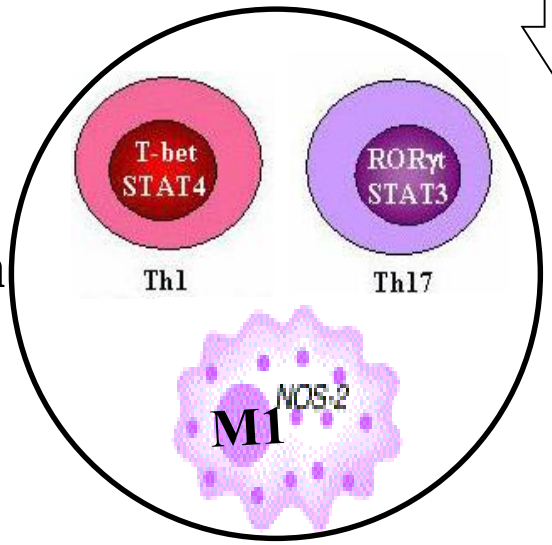
Not scolicidal

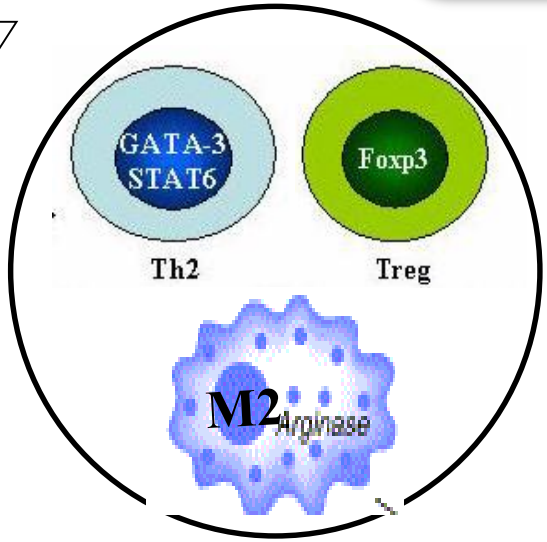


+ IL-10

After 20h

Inflammation





Regulation of inflammation

Host defense

Parasite survival

CONCLUSION 3

The existence of a parasitic immune evasion mechanism is therefore clearly established

??

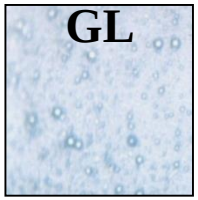
Which specific component of the hydatid cyst is responsible for this immune escape ?

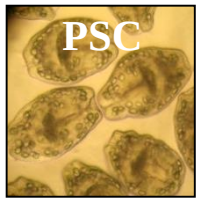
SCIENTIFIC APPROACH

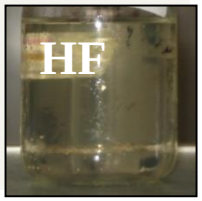
Part 3

Evaluate the implication of different components of hydatid cyst in the regulation of these pathways

***Ex vivo* on PBMCs cultures or PBMC-PSC cocultures stimulated by different cyst part**







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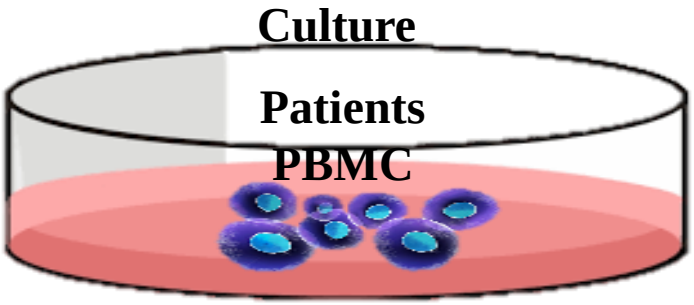
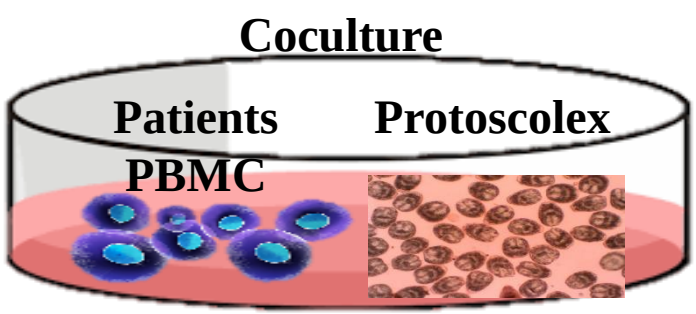


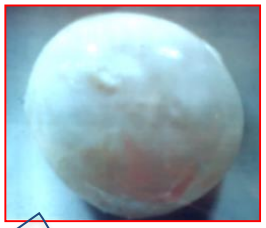


CONCLUSION 4

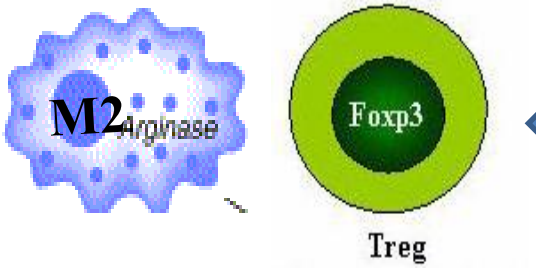
LL:

- ✓ Accellular
- ✓ Rich in carbohydrates
- ✓ Physical protection of the cyst

Hydatic cyst



Regulation of inflammation



Induces

+

These parasite survival strategies involve: the LAMINATED LAYER (LL)

Acta Tropica 149 (2015) 186–194

Contents lists available at ScienceDirect

Acta Tropica

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

A protective effect of the laminated layer on *Echinococcus granulosus* survival dependent on upregulation of host arginase

Manel Amri, Chafia Touil-Boukoffa*

Steers et *al*., 2001

Parasite Immunology, 2001: **23:** 411–417

In-vitro susceptibility of hydatid cysts of *Echinococcus granulosus* to nitric oxide and the effect of the laminated layer on nitric oxide production

N.J.R.STEERS, M.T.ROGAN & S.HEATH

Division of Biological Sciences, School of Environment and Life Sciences, University of Salford, Salford, UK

TEAM PUBLICATIONS

Acta Tropica 149 (2015) 186–194

Contents lists available at ScienceDirect

Acta Tropica

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

A protective effect of the laminated layer on *Echinococcus granulosus* survival dependent on upregulation of host arginase

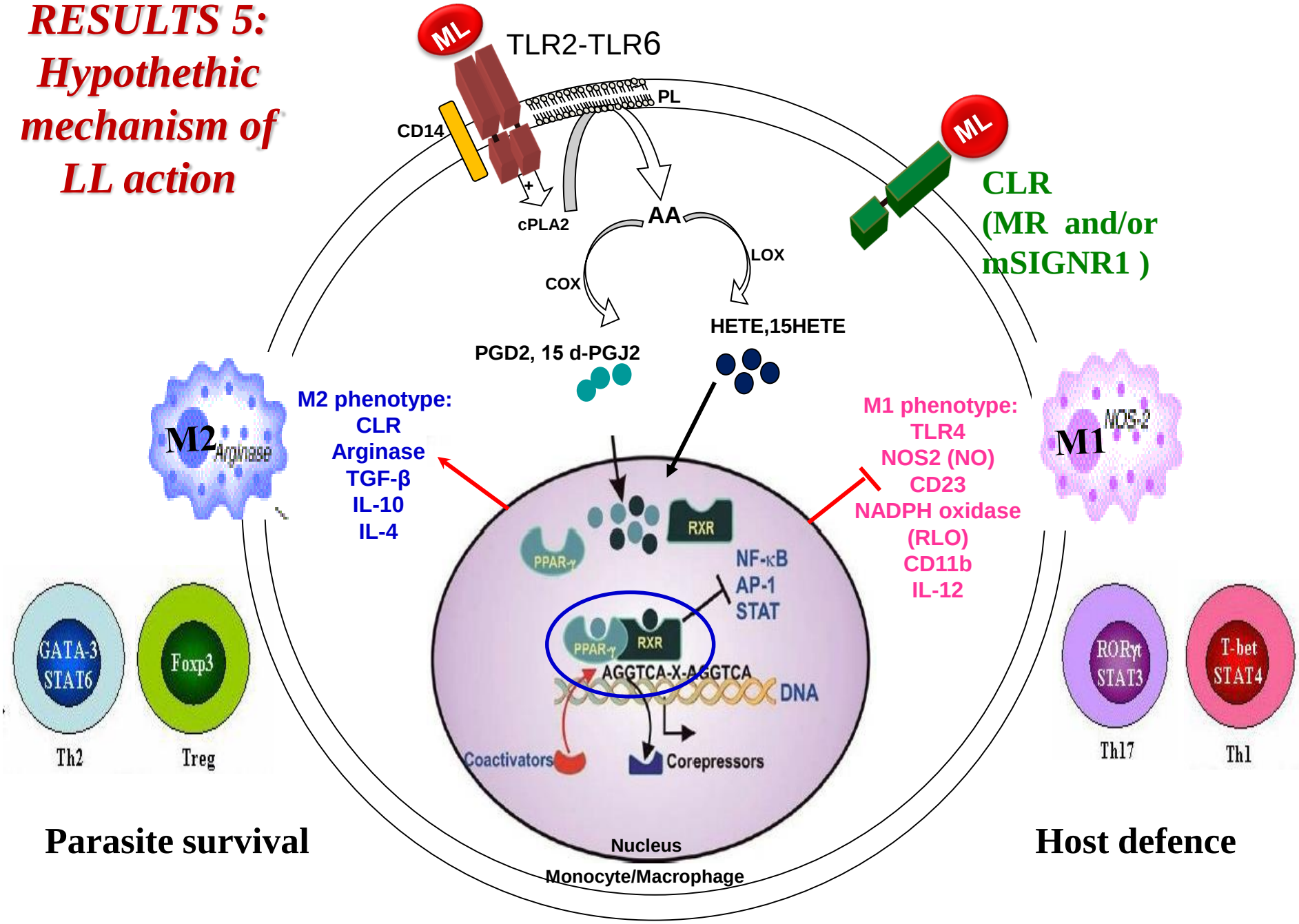
Manel Amri, Chafia Touil-Boukoffa *

Acta Tropica
Volume 218, June 2021, 105886

In vitro immunoregulatory activity and anti-inflammatory effect of *Echinococcus granulosus* laminated layer

Sara Benazzouz [a], Manel Amri [a], Junhua Wang [b, c], Samia Bouaziz [a], Fahima Ameur [a], Sara Djebbara [a], Karima Achour [d], Bruno Gottstein [b, c], Chafia Touil-Boukoffa [a]

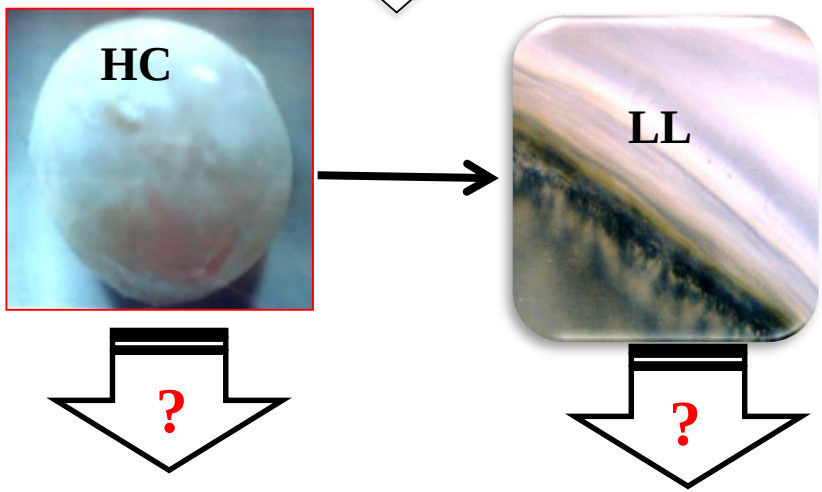
RESULTS 5: Hypothethic mechanism of LL action



CONCLUSION 5

All of these data suggest

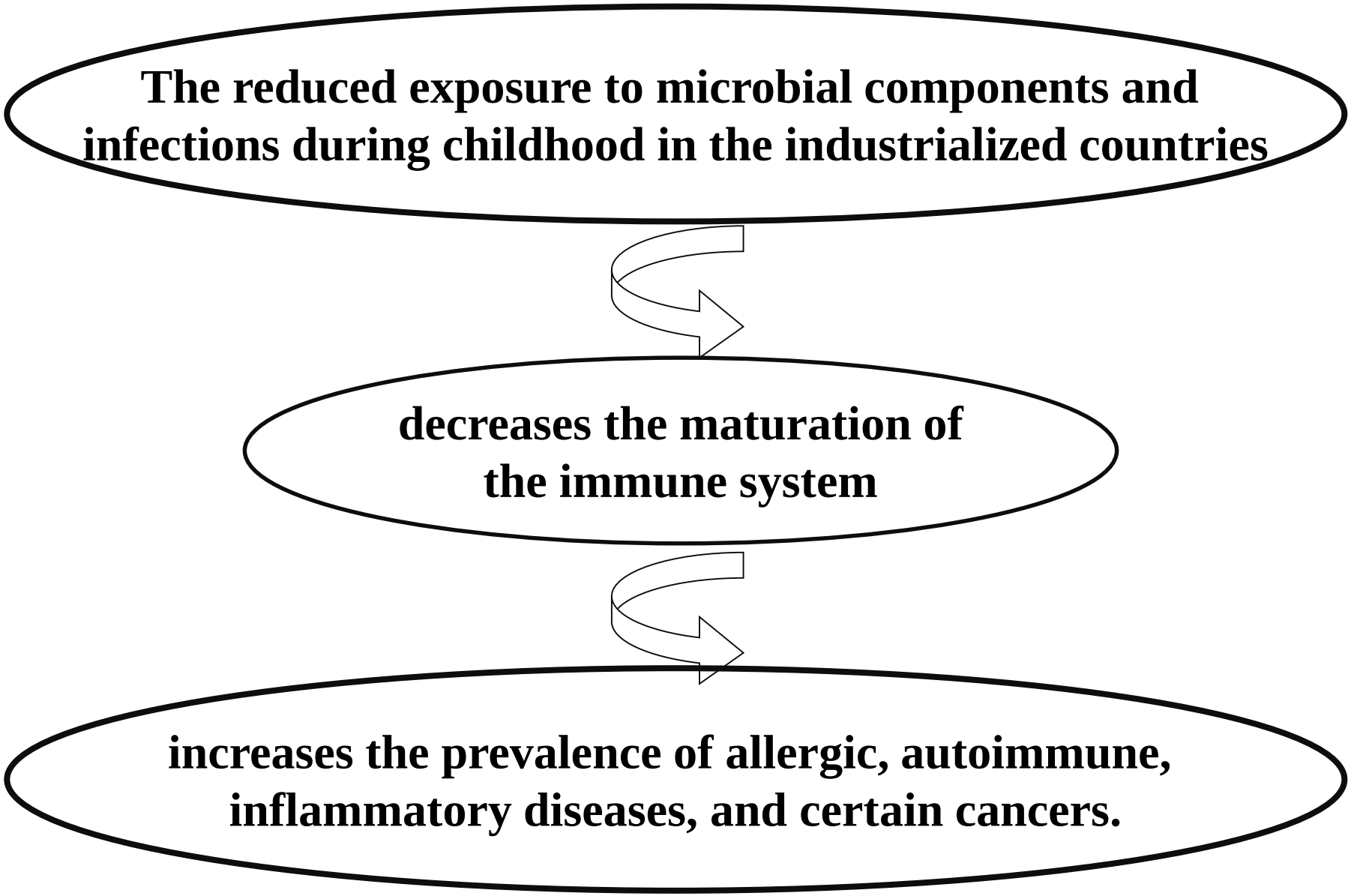
LL exhibits an anti-inflammatory & immunoregulatory effect



Do they act as protective or therapeutic agents in inflammatory diseases?

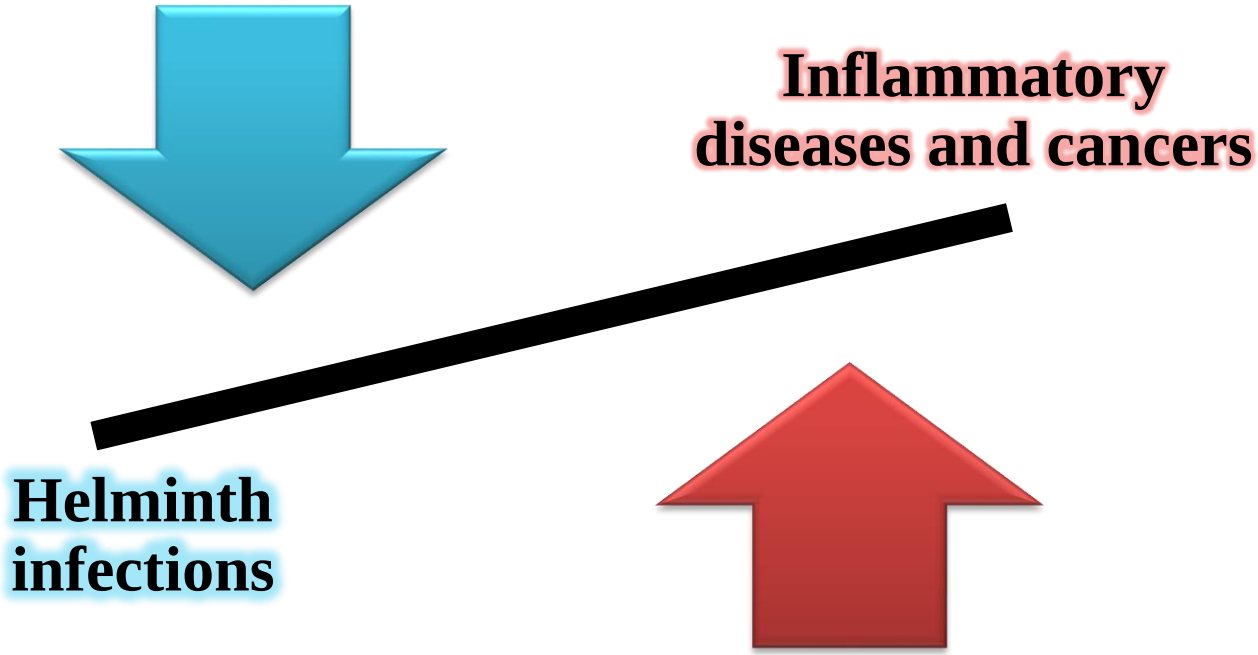
Notion: THE "HYGIENE HYPOTHESIS"

THE "HYGIENE HYPOTHESIS"



Theory based on several epidemiological studies

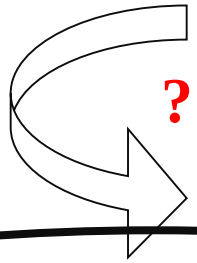
Which highlights the existence of an inverse correlation between the incidence of certain inflammatory and autoimmune diseases and exposure to helminths



Clinical trials using helminth therapy for the treatment of autoimmune diseases

Pathologies	Helminth species	Treatment	Observed outcomes	References
Crohn's disease	***Necator americanus***	*Inoculation of larvae at week 0 (n = 9). Reinoculation between weeks 27 and 30 (n = 5).	*No serious adverse events. *5 patients from the first inoculation entered remission by week 45.	(Croese et al., 2006)
Multiple sclerosis	**Multiple species**	Infection of 12 patients.	*Patients infected with helminths experienced fewer exacerbations. *A significant increase in IL-10 and TGFβ and a decrease in IL-12 and IFNγ were observed in autoreactive cells.	(Correale et al., 2007)
Celiac disease	***Necator americanus***	Larval inoculation at weeks 0 and 4, followed by a progressive low-dose gluten challenge in 12 subjects.	*No serious adverse events. *10 subjects tolerated a low-dose gluten challenge.	(Croese et al., 2015)
Type 2 diabetes	***Strongyloides stercoralis***	Infection of 60 patients.	Decrease in plasma levels of cytokines and chemokines.	(Rajamanickam et al., 2020)

HYGIENE HYPOTHESIS / *Echinococcus granulosus*



We hypothesize that the immunomodulation induced by *Echinococcus granulosus* or its larval components offers a protective and/or curative potential against chronic inflammatory diseases, autoimmune disorders, allergies, and cancers."

Several publications support our hypothesis

Protective or Therapeutic effect of *Echinococcus granulosus* on inflammatory and auto-immune diseases

- ✓ Moradi, K., et al. (2025). Protective effects of **hydatid cyst fluid** on inflammation and tissue damage in rat model of **type 1 diabetes**. Cell Communication and Signaling, 23(1), 172.
- ✓ Samei, A., & Khedri, M. (2025). Immunotherapeutic Potential of *Echinococcus granulosus* **Hydatid Cyst** Antigens in **Autoimmune Disease and Allergy.** Iranian Journal of Allergy, Asthma and Immunology, 24(3), 259-267.
- ✓ Hajizadeh, M., et al. (2024). Modulatory effects of **hydatid cyst fluid** on a mouse model of **experimental autoimmune encephalomyelitis** (EAE). Veterinary Sciences, 11, 34.
- ✓ Bao, J., et al. (2022). *Echinococcus granulosus* sensu stricto and **antigen B** may ameliorate **experimental inflammatory bowel disease** by shifting macrophage polarization. Parasites & Vectors, 15, 1–14
- ✓ Wang, H., et al. (2014). ***Echinococcus granulosus*** **infection** reduces **airway inflammation** in mice likely through enhancing IL-10 and down-regulation of IL-5 and IL-17A. Parasites & Vectors, 7, 522.

Several publications support our hypothesis

Anti-cancer effect of *Echinococcus granulosus* (REVIEW)

- ✓ Sadr, S, Borji, H, (2023), ***Echinococcus granulosus*** as a promising therapeutic agent **against triple negative breast cancer**, Current Cancer Therapy Reviews
- ✓ A Shojaeian, N Barati, S Motavallihaghi, (2023). Investigating the Inhibitory Effects of **Hydatid Cyst** Fluid and its Antigens on **Cancer Progression**: A Review,
- ✓ Almeida, P., et al. (2020). The **hydatid cyst** and tumor microenvironment: cross-talk and potential antitumor effects. Cancer Immunology Research, 8, 1123–1132
- ✓ Guan W, et al, (2019). Employing Parasite **Against Cancer**: A Lesson From the Canine Tapeworm ***Echinococcus granulocus*.** Front Pharmacol. 2019 Sep 25;10:1137.

SCIENTIFIC APPROACH

Part 1

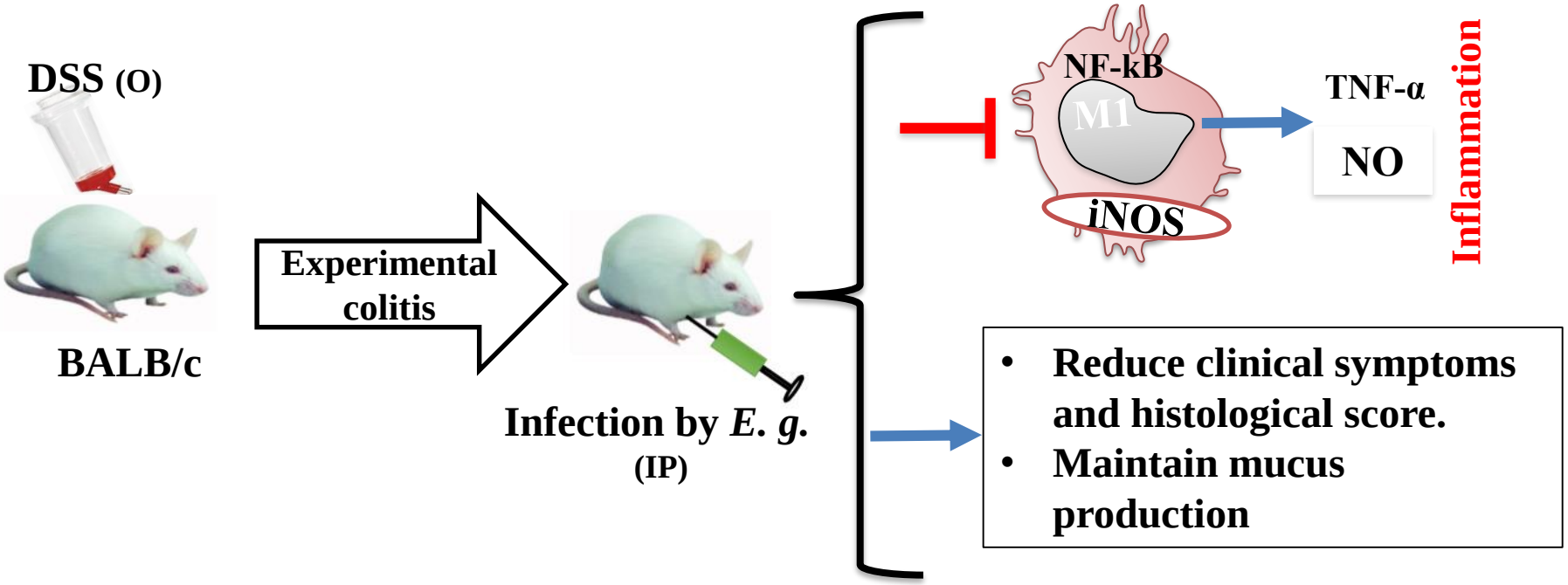
Confirm the application of « the hygiene theory » to our study model

Evaluate the effect of

***Echinococcus granulosus* infection**

during experimental colitis

Echinococcus granulosus infection protects mice against DSS-induced colitis



(Khelifi *et al.*, 2017)

ELSEVIER

Contents lists available at ScienceDirect

Acta Tropica

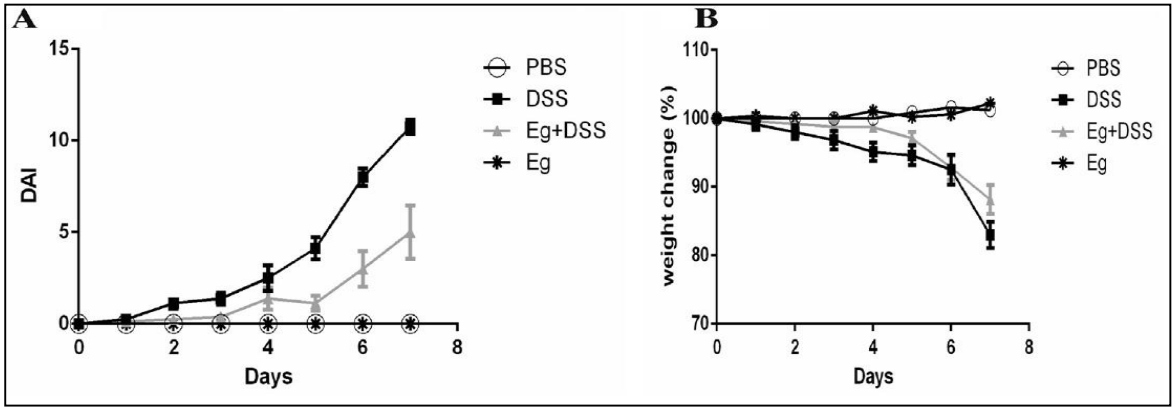
journal homepage: www.elsevier.com/locate/actatropica

Immune-protective effect of echinococcosis on colitis experimental model is dependent of down regulation of TNF-α and NO production

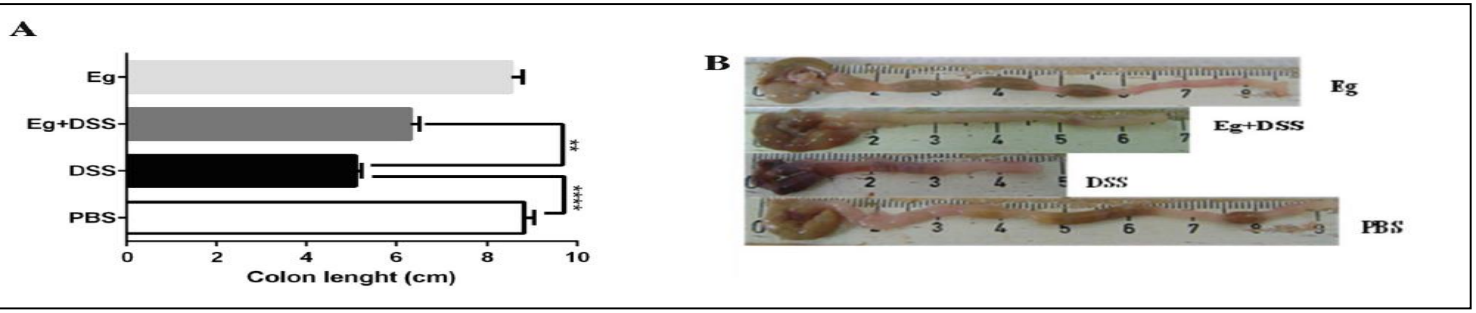
Lila Khelifi, Imene Soufli, Moussa Labsi, Chafia Touil-Boukoffa*

Laboratory of Cellular and Molecular Biology, Department of Biology, University of Sciences and Technology Houari Boumediene, Algiers, Algeria

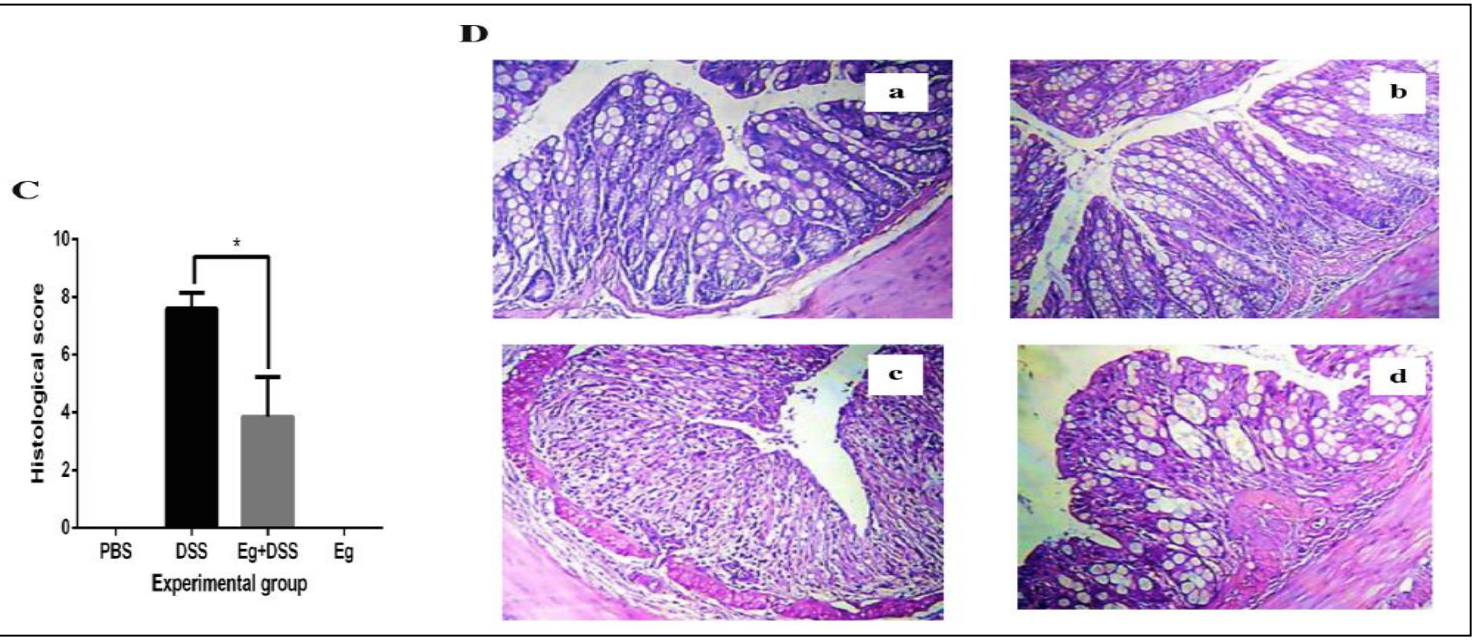
Disease activity indice
DSS: ↑↑
Eg +DSS: ↓↓



Mice weight
DSS: ↓↓
Eg +DSS: ↑↑



Colon size
DSS: ↓↓
Eg +DSS: ↑↑



Histological score
DSS: ↑↑
Eg +DSS: ↓↓

SCIENTIFIC APPROACH

Part 2

Confirm that the Laminated layer (LL) is (at least partially) the component responsible for the observed effects.

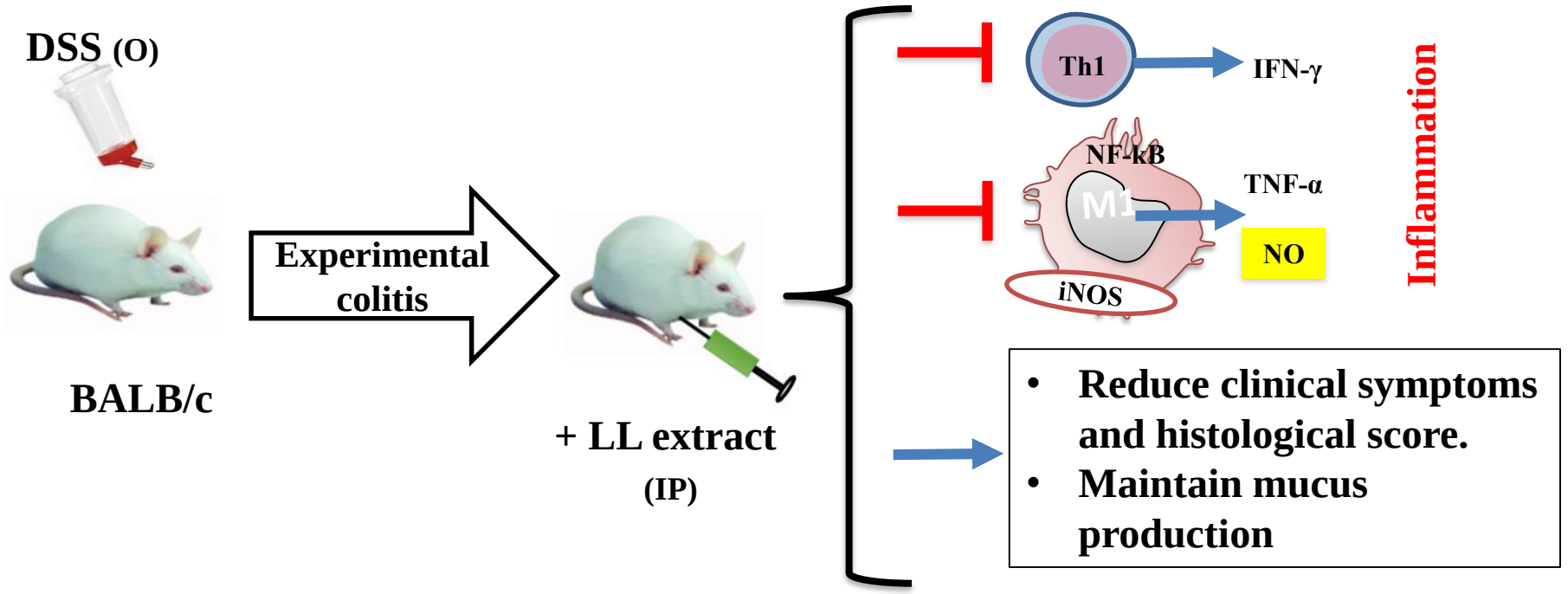
Evaluate the effet of LL during several diseases:

1/ Experimental colitis

2/ Experimental allergic asthma

3/Experimental autoimmune uveitis

The laminated layer (LL) protects mice against DSS-induced colitis



(Soufli *et al.*, 2015)

Soufli *et al. Journal of Inflammation* (2015) 12:19
DOI 10.1186/s12950-015-0063-6

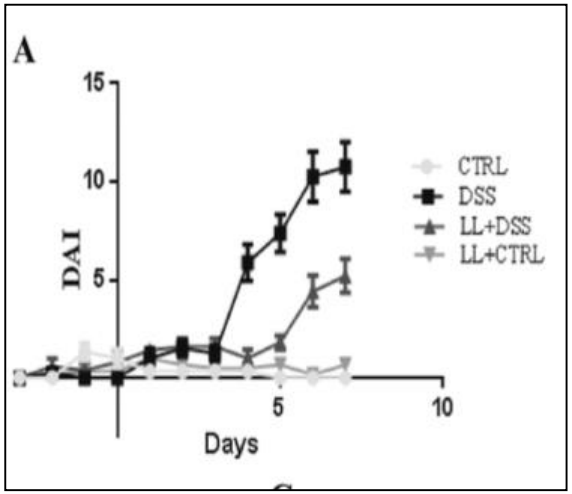
JOURNAL OF INFLAMMATION

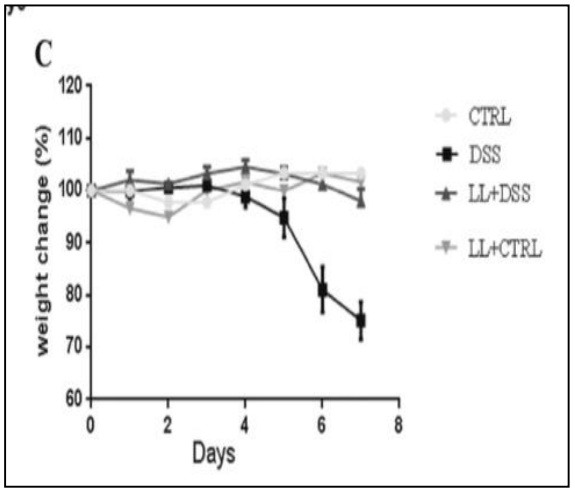
RESEARCH Open Access

Crude extract of hydatid laminated layer from Echinococcus granulosus cyst attenuates mucosal intestinal damage and inflammatory responses in Dextran Sulfate Sodium induced colitis in mice

Imene Soufli[1], Ryma Toumi[1], Hayet Rafa[1], Manel Amri[1], Moussa Labsi[1], Lila Khelifi[1], Ferdinando Nicoletti[2] and Chafia Touil-Boukoffa[1*]

Disease activity indice
DSS: ↑↑
LL +DSS: ↓↓

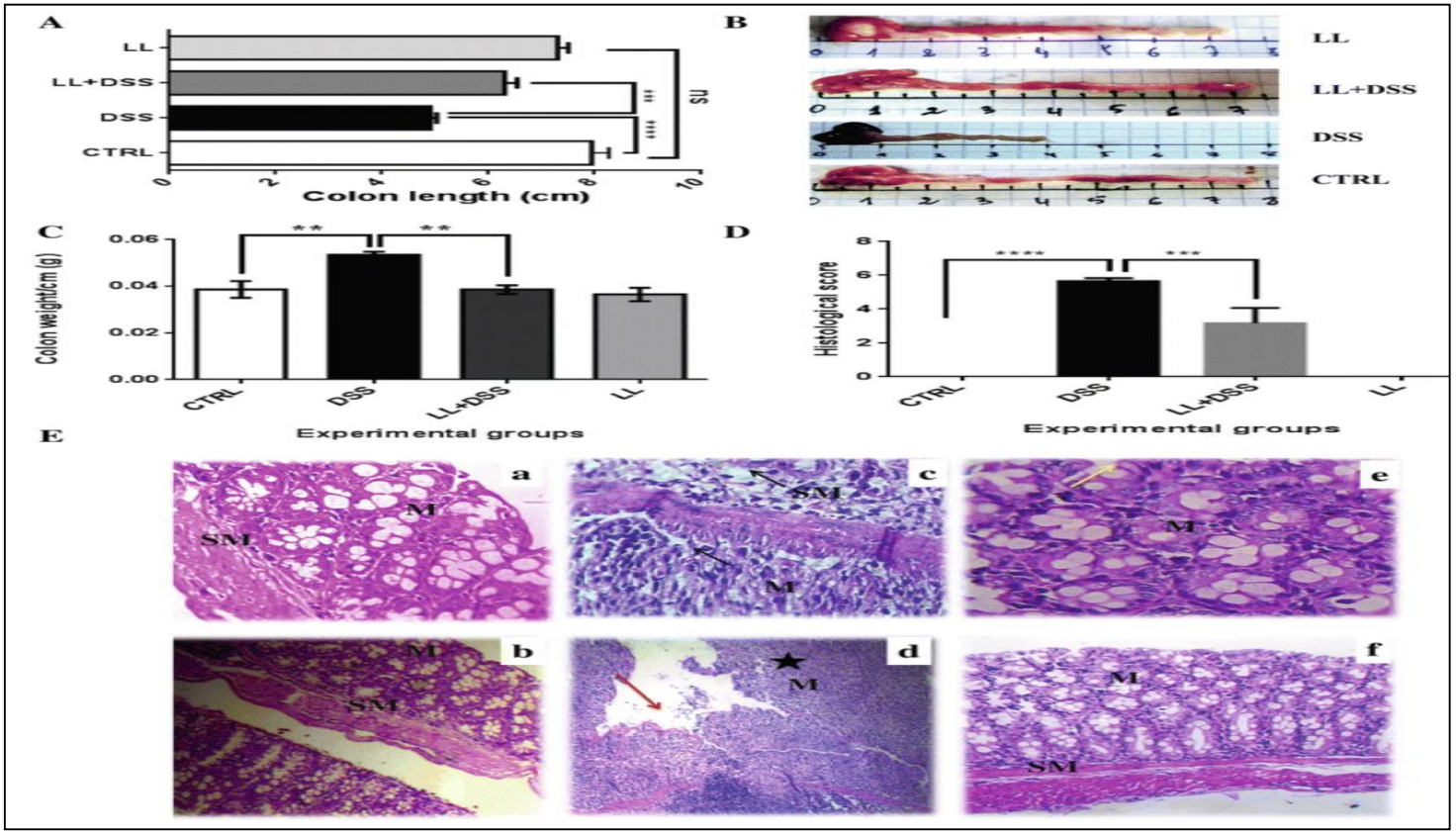




Mice weight
DSS: ↓↓
LL +DSS: ↑↑

Colon size
DSS: ↓↓
LL +DSS: ↑↑

Histological score
DSS: ↑↑
LL +DSS: ↓↓



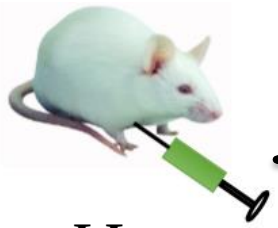
The laminated layer (LL) protects mice against ovalbumin-induced allergic asthma

Ovalbumine

(IP THEN aErosol)

BALB/c

Experimental allergic asthma



+ LL extract

(IP)

Th1 → **IFN-γ**

Th17 → **IL-17A**

M1 → **IL-1β**, **NO**

Inflammation

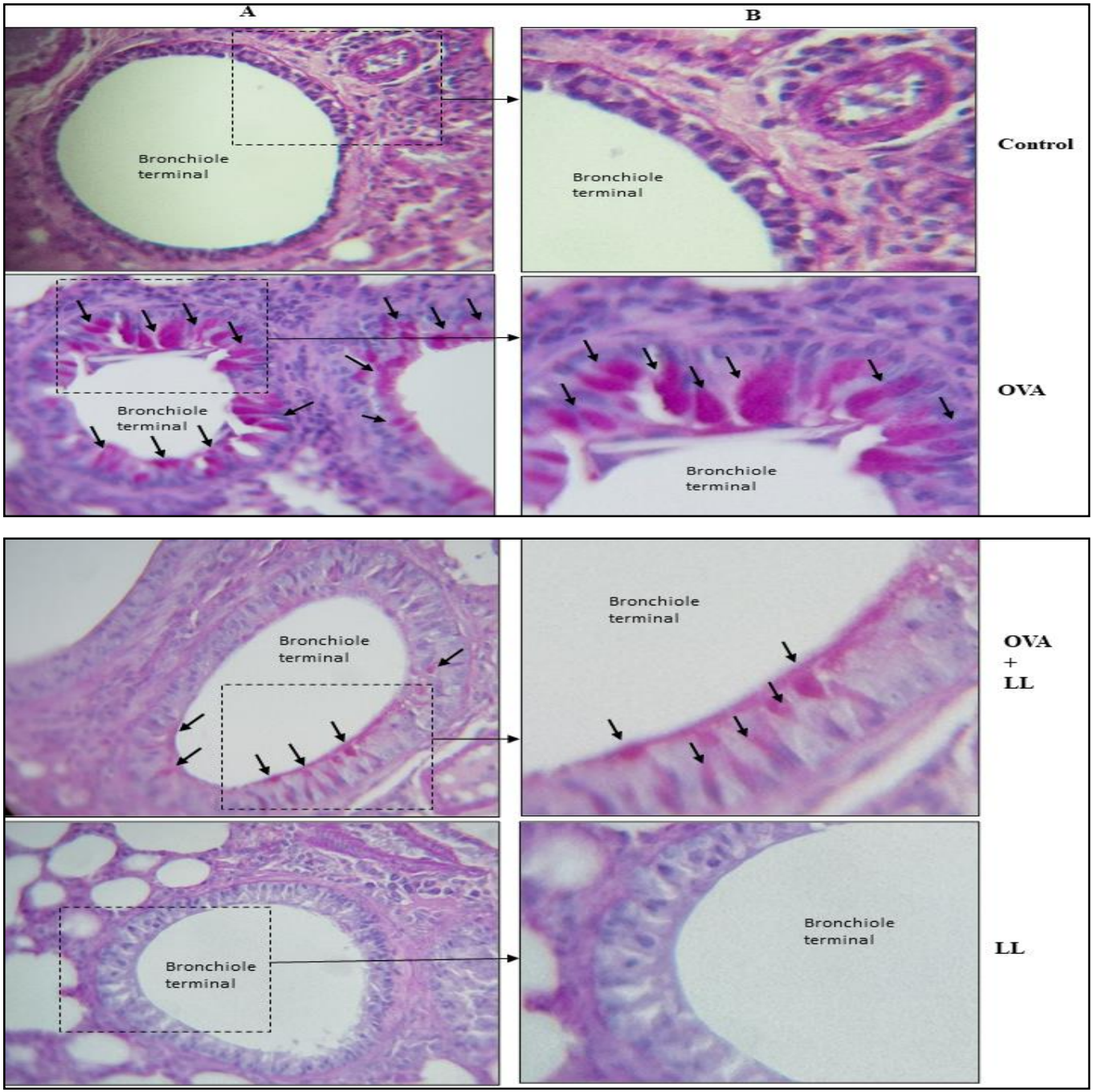
Treg → **TGF-β**, **FOXP3**

Inflammation regulation

Reduces

- **Tissus damage**
- **Cells infiltration**
- **Mucus production by lung goblet cells**

(Benazzouz *et al.*, in writing)



Reduction by LL of:

*** The inflammatory infiltrate**

*** Mucus production**

*** Goblet cell hyperplasia**

during ovalbumin-induced asthma

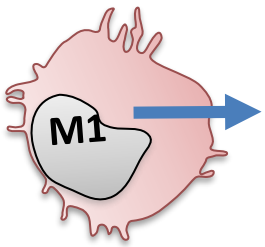
The laminated layer (LL) protects mice against autoimmune uveitis

Bovine Retinal Extract (EBR) + CFA (SC)

Wistar rat

Experimental autoimmune uveitis

+ LL extract (IP)



TNF-α

NO

Oxydative stress (MPO, MDA, Cat, SOD)

Inflammation

Reduces

- Retinal histological damage
- Cells infiltration

(Djebarra *et al.*, 2025)

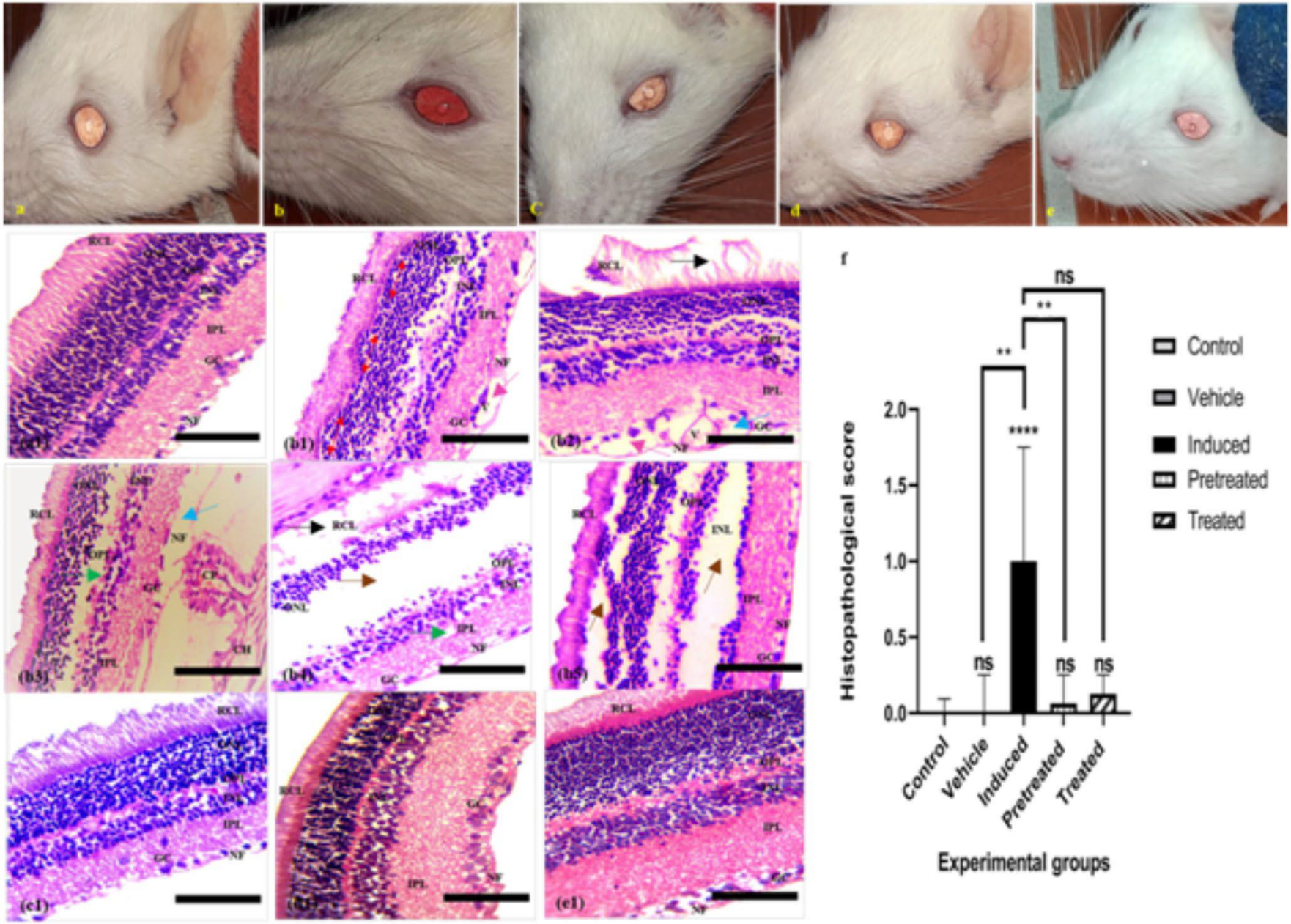
Acta Parasitologica (2025) 70:34
https://doi.org/10.1007/s11686-024-00944-6

RESEARCH

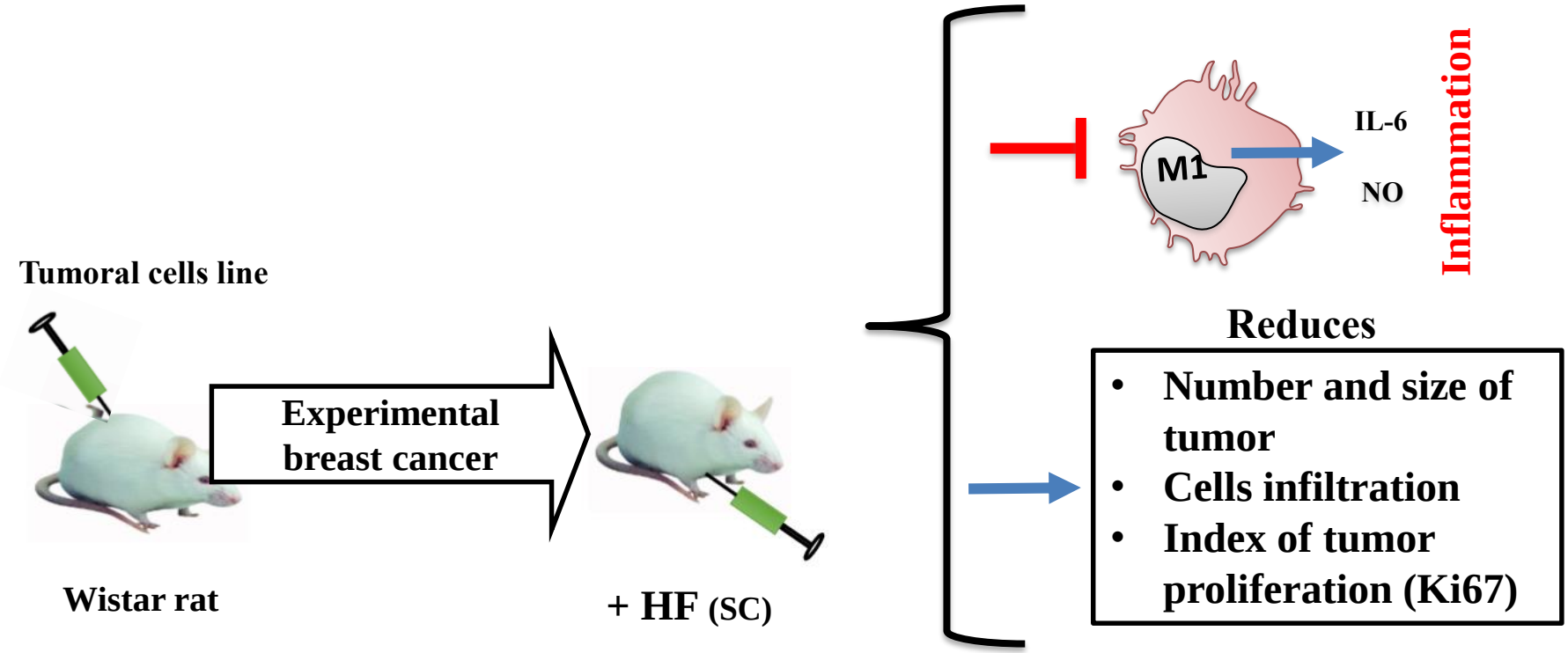
Laminated Layer Extract from *Echinococcus Granulosus* cyst Attenu Ocular Damages and Inflammatory Responses in an Experimental Autoimmune Uveitis Model

Sara Djebbarra[1] · Houda Belguendouz[1] · Imene Soufli[1] · Leila Hannachi[2] · Fahima Ameur[1] · Sara Benazzouz[3] · Sarra Benkhelifa[1] · Malika Terrab[4] · Karima Achour[5] · Zine-Charaf Amir[6] · Manel Amri[1] · Chafia Touil-Boukoffa[1]

Reduction of Retinal damage



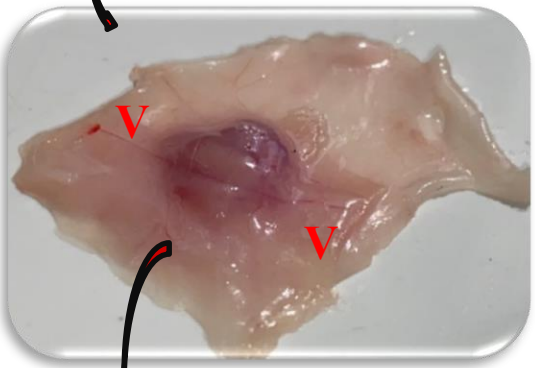
The Hydatid fluid (HF) protects mice against Cell Line-Derived Xenograft (CDX)-induced breast cancer

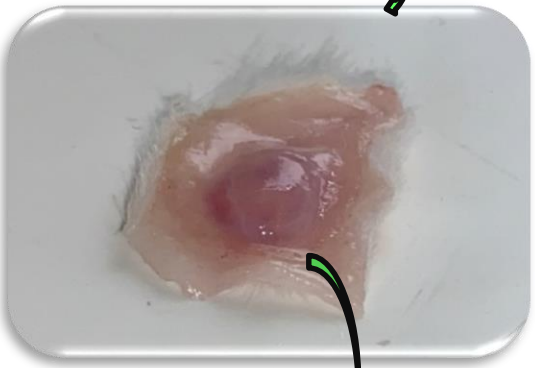


(Boussa *et al.*, not yet published)

Untreated rat

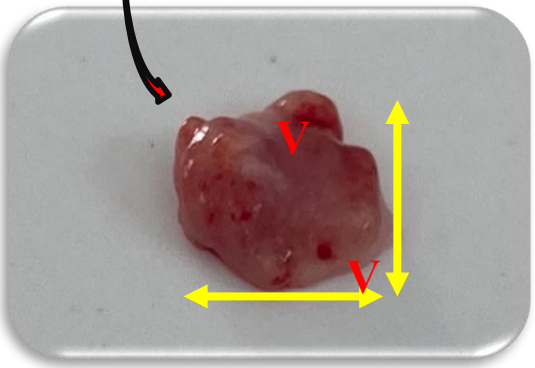
Treated rat

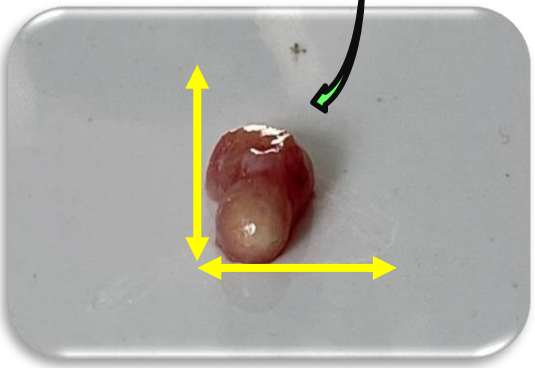




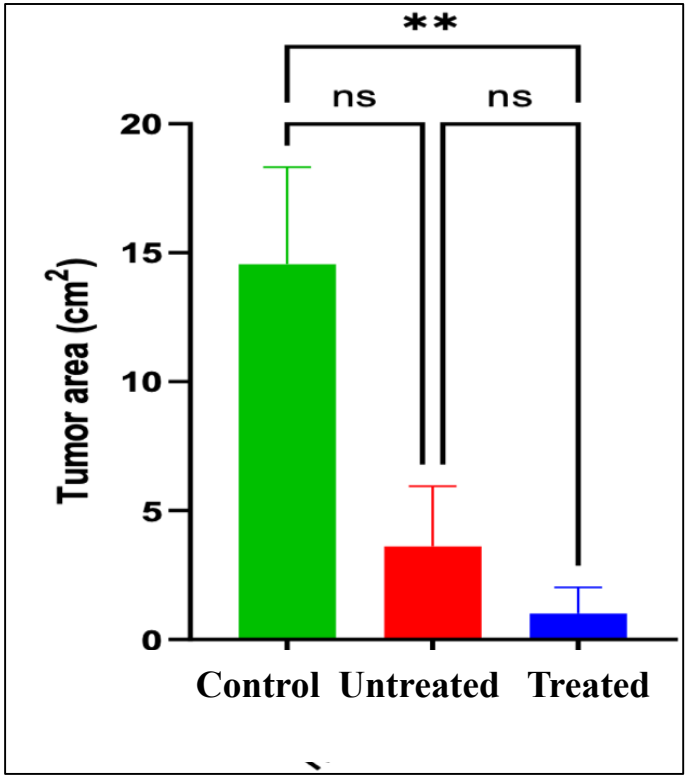
Untreated rat

Treated rat





HF treatment decreases:
1/ The number of the tumor
2/ The size of the tumor (↔)
2/ Vascularization (V)



CONCLUSION

Our work on **Cystic Echinococcosis (CE)** has enabled us to draw a complex portrait of the host-parasite interactions, with implications that extend far beyond the field of parasitology itself.

1. **Immune equilibrium** between the parasite (*Echinococcus granulosus*) and its host,
2. **The potential of cytokines as prognostic marker** for patient follow-up.

3, The key role of the laminated layer in the parasite's survival strategies.

4, Therapeutic potential of hydatid cyst components, particularly the **Laminated layer** in experimental models of various pathologies, including:

- **Inflammatory diseases**
- **Autoimmune conditions**
- **Cancers**

Ongoing research in our lab

Highlight the potential immunoregulatory, protective and/or curative effect of LL, HF, and excretory-secretory products of PSC on other pathologies (PR, CCR, ..)

***Ex vivo* during human CE (PBMC, biopsies, …)**

***In vivo* in experimental models:**
1) Freud's adjuvant-induced arthritis
2) DSS+DMH-induced colorectal cancer mouse model
3) Breast cancer

Identify and characterize the biomolecules responsible for these effects

Proteomic analysis
(Zeghir-Bouteldja et *al.*, 2017 et 2022)

*Hydatid fluid

ELSEVIER

Acta Tropica
Volume 171, July 2017, Pages 199-206

Comparative proteome profiling of hydatid fluid from Algerian patients reveals cyst location-related variation in *Echinococcus granulosus*

Razika Zeghir-Bouteldja [a, b] ✉, Andy Polomé [c] ✉, Sabrina Bousbata [c] ✉, Chafia Touil-Boukoffa

* Laminated layer

RESEARCH ARTICLE

IDENTIFICATION OF PROTEINS OF LAMINATED LAYER OF *ECHINOCOCCUS GRANULOSUS*: INTERFACE AMONG HOST AND PARASITE

Razika Zeghir-Bouteldja[1,2*], Chafia Touil-Boukoffa[2]

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All our **national and international collaborators**
All **Patients**

THANK YOU FOR YOUR ATTENTION