Simposio
Estrategias inmunológicas, farmacológicas, antiparasitarias y antitumorales en torno a *Trypanosoma cruzi*

Tissue responses again parasites and tumor invasion: differences and similarities in extracellular matrix reorganization

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The extracellular matrix is a key regulator of cell and tissue function. Traditionally, the extracellular matrix is considered as a physical scaffold that binds cells and tissues together (5). However, the extracellular matrix is a dynamic structure that interacts with cells and generates signals through feedback loops to control the behavior of cells. Thus, extracellular matrix macromolecules are bioactive and modulate cellular events such as adhesion, migration, proliferation, differentiation, and survival (3). Additionally, extracellular matrix molecules are strictly organized and this organization determines the bioactivity of it. Even minor alterations in a single extracellular matrix component can lead not only to altered physicochemical properties of tissues but also to changes in cellular phenotype and cell-matrix interactions. It has been proposed that these changes in extracellular matrix structure and bioactivity in tissue function ultimately lead to development of disease (6).

Parasites and neoplastic cells, during tissue invasion or tumor dissemination, modify extracellular matrix in order to modulate host immune responses and facilitate the mobilization inside the tissues (2,4). *Trypanosoma cruzi*, the causative agent of Chagas disease, induces changes in extracellular matrix in different tissues, for example in myocardium (7) and in human placental chorionic villi (4). Neoplastic cells modify host microenvironment that undergoes extensive changes during the evolution and progression of cancer. This involves the generation of cancer-associated fibroblasts, which, through release of growth factors and cytokines, lead to enhanced angiogenesis, increased tumor growth and invasion. The altered fibroblast phenotype also contributes to the development of an altered extracellular matrix. The inflammatory infiltrate associated with many solid tumors also modulates tumor function, having both anti- and pro-tumor effects (1).

We have analyzed changes in extracellular matrix induced by *T. cruzi* in human placental chorionic villi as well as those induced in tumor stroma and neighboring connective tissue by oral squamous cell carcinoma and by salivary papillary cystadenocarcinoma.

Extracellular matrix changes induced by *Trypanosoma cruzi* in human placental chorionic villi

The human placenta is classified as a hemochorial villous placenta in which the free chorionic villi are the functional units. These chorionic villi are formed by the trophoblast and the villous stroma. The trophoblast is formed by a single multinucleated cell layer (syncytiotrophoblast) which contacts maternal blood in the intervillous space, and by the cytotrophoblast which contains replicating progenitor cells. The trophoblast is separated by a basal lamina from the villous stroma, which is connective tissue containing vascular endothelium, fibroblasts, and macrophages. Trophoblast, basal laminae, and villous stroma with endothelium of fetal capillaries form the placental barrier that must be crossed by different pathogens, including *T. cruzi*, in order to infect the fetus during vertical transmission (4).

Trypanosoma cruzi induces syncytiotrophoblast destruction and detachment, selective disorganization of basal lamina and disorganization of collagen I in the connective tissue of villous stroma. These effects can be observed in placentas of mothers with chronic asymptomatic Chagas disease as well as in *ex vivo* infected chorionic villi explants. In the chorionic villi explants these effects are a function of the number of parasites used for the infection. The destruction of the extracellular matrix is product of activation of proteases of the parasite (cruzipain) and matrix metalloproteases (MMP) of the placental tissue. *Trypanosoma cruzi* induces expression and activity of MMP-2 and MMP-9 in chorionic villi explants. Inhibition of the proteases prevents partially the extracellular matrix destruction induced by the parasite.

It has been proposed that extracellular matrix alterations produced by *T. cruzi* not only promote its motility in tissues and its entrance into cells, but also alter the presence of cytokines and chemokines, which in turn permit this parasite to modulate and escape both the inflammatory response and the immune response (4,7). Alternatively, these changes in extracellular matrix function may be part of local placental defense mechanisms, which could explain both the low presence of parasites in the placenta and the low incidence of congenital Chagas disease.

Extracellular matrix changes in neoplastic lesions with different degree of malignancy

Cancer cell migration and invasion into adjacent connective tissue depends on extracellular matrix, which provides a physical scaffold for cell adhesion and migration. Proteolysis of the extracellular matrix regulates cellular migration by modifying the structure of the extracellular matrix scaffold and by releasing extracellular matrix fragments with biological functions. Extracellular matrix proteolysis is therefore tightly controlled in normal tissues but typically deregulated in tumors (5). Changes in extracellular matrix in areas of invasive tumor front are used in some systems to classify neoplastic lesion of epithelia origin, like the Bryne's multifactorial grading system for the invasive tumor front (8).

In highly invasive (poor differentiated) oral squamous cell carcinoma destruction of basal lamina and disorganization of collagen I in adjacent connective tissue is present. Contrarily, in low grade papillary cystadenocarcinoma and well differentiated oral squamous cell carcinoma only in some focal areas a slight discontinuity can be observed and the collagen I in the adjacent connective tissue is well organized.

Therefore the organization of the extracellular matrix can prevent or facilitate the tumor invasion and dissemination. Poor differentiated tumors modify the extracellular matrix in order to facilitate the invasion process. Contrarily, well differentiated tumors are not able to modify the extracellular matrix and the tissue can impair the invasion and dissemination of the neoplastic lesion.

In summary, parasite and tumors induces extracellular matrix responses that can facilitate or impair tissue invasion. The extracellular matrix response depends on the type of aggression (parasite, type of tumor) as well as of the type of tissue that is being challenged.

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Trypanosoma cruzi calreticulin: a pleiotropic molecule in the host/parasite interplay

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Trypanosoma cruzi calreticulin (TcCRT) is a parasite virulence factor that participates in important aspects of the *T. cruzi* interactions with its vertebrate host. Among the known parasite surface molecules participating in infectivity, TcCRT, after being translocated from the endoplasmic reticulum to the area of flagellar emergence, recruits complement C1 and interferes with the ability of the associated C1r and C1s serine proteases to activate the central component C4. By recruiting C1, parasite surface TcCRT also promotes early C1-dependent phagocytosis, a phenomenon reminiscent of what human apoptotic cells do.

In spite of the evolutionary distance between trypanosomatids and mammals, the phagocytic removal of apoptotic and tumor cells by macrophages and dendritic cells has also been proposed as strongly dependent on C1 that is recruited by membrane-translocated vertebrate CRT. C1 affinity for antigen-aggregated immunoglobulins, and TcCRT immunogenicity, will also contribute to a state of self-sustained *in vivo* parasite infectivity.

The interaction between TcCRT and C1 can be partially reversed by $F(ab´)_2$ fragments (bivalent antigen-specific immunoglobulin fragments, devoid of their Fc portions and thus unable to bind the first complement component C1 prepared from anti-TcCRT Igs, thus reversing the TcCRT-promoted infectivity.

On the other hand, externalized TcCRT, by itself, will bind to endotheliocytes from both arterial and venous emerging capillaries, as tested in a variety of assays in four vertebrate species. Inhibition of angiogenesis will follow, a phenomenon that could explain the capacity of this molecule to inhibit tumor growth *in vivo* and, perhaps the antitumor effects reported for the parasite infection in experimental *in vivo* set ups. All these properties map to the TcCRT N-terminal domain.

The combined anti-angiogenic and anti-complement TcCRT effects may be anti-inflammatory, thus inhibiting the immune response against the parasite.

Considering these results altogether, it could be speculated that the interactions among TcCRT, complement, and endothelial cells are calibrated evolutionary adaptations aimed at protecting both the parasite and the host, thus promoting long-term interplays.

These findings open possibilities for the development of new experimental anti-tumor strategies, especially if we consider that TcCRT displays stronger anti-angiogenic and anti-tumor effects than its human counterpart. Whether the anti-angiogenic properties were consolidated first in the parasite chaperone molecule, and HuCRT conserved some of these properties as an evolutionary relic or, alternatively, the parasite hijacked this activity from its vertebrate host, remains an open question. Although *in vivo*, both *T. cruzi* infection and TcCRT treatment, inhibit tumor development, it is not possible at present to causally associate both phenomena (i.e., that *T. cruzi* infection inhibits tumor growth through the anti-angiogenic *T. cruzi* capacity).

In synthesis, it could be proposed that not all consequences of host-parasite interactions are deleterious to the former, and that the parasite has developed cellular and molecular strategies that could benefit the host, with indirect benefits for the aggressor.

Suggested readings

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Disfunción endotelial en la cardiopatía chagásica crónica: un enfoque terapéutico de la enfermedad de Chagas

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Aproximadamente, 10 a 30 % de los pacientes infectados con *Trypanosoma cruzi* desarrollan manifestaciones típicas de la fase crónica, siendo la más importante la cardiopatía chagásica, principal causa de muerte en esta enfermedad. La cardiopatía chagásica crónica se presenta con síntomas y signos que derivan de una falla cardiaca, la cual es usualmente biventricular y, además, se presenta con arritmias complejas, tromboembolia y muerte súbita (1). Al cabo de cinco años de establecido el diagnóstico, la mortalidad alcanza el 50 % de los pacientes infectados.

La fisiopatología de la cardiopatía chagásica crónica es compleja y multifactorial. Sin embargo, se han postulado cuatro mecanismos principales que explican esta alteración:

i) daño al miocardio, dependiente del parásito, daño al miocardio, mediado por la respuesta inmunitaria,
ii) disautonomía cardiaca, y
iv) anormalidades microvasculares e isquemia (2).

Evidentemente, la persistencia del parásito puede provocar daño directo en los miocitos, pero también provoca una respuesta inmunológica e inflamatoria permanentes que, necesariamente, alteran la arquitectura cardiaca por una miocarditis persistente.

Por otro lado, el sistema autónomo está alterado debido a la presencia de autoanticuerpos contra receptores adrenérgicos y colinérgicos del miocardio en pacientes con enfermedad de Chagas, lo que provoca alteraciones fisiológicas, morfológicas, enzimáticas y moleculares, y conduce a denervación parasimpática y activación simpática, lo que genera la ausencia de mecanismos que regulen el ritmo cardiaco.

Finalmente, en diversos estudios se ha observado que hay anormalidades microvasculares que incluyen constricción vascular focal, proliferación microvascular y trombos oclusivos de plaquetas en arterias coronarias, que llevan a isquemia. Es más, en corazones chagásicos se ha observado distribución focal de necrosis celular y fibrosis intersticial, similar a lo observado en modelos experimentales de isquemia y reperfusión.

Existe una relación directa entre las plaquetas y el endotelio inflamado cuando éste es activado. La respuesta endotelial a la infección por *T. cruzi* incluye activación del factor de necrosis NF-κB, expresión de moléculas de adhesión (E-selectina, VCAM-1, ICAM-1) y aumento en la sensibilidad celular del endotelio a la infección, además de lo cual, favorece la agregación plaquetaria en la superficie endotelial y la trombosis microvascular.

En el proceso de agregación plaquetaria, también intervienen directamente prostanoides, como el tromboxano A_2 (2). Sin embargo, la participación de prostaglandinas, como la prostaglandina E_2

(PGE_2), en la fisiopatología de la enfermedad de Chagas es más compleja, pues no sólo participan en la contención de la infección, sino también en procesos de evasión de la respuesta inmunitaria y en el proceso inflamatorio propio de la miocarditis chagásica. Es más, el tromboxano por sí mismo es vasoconstrictor y proinflamatorio y, por ello, puede contribuir al daño isquémico y a la cardiopatía (3).

Los estudios realizados en nuestro laboratorio han revelado que en un modelo de ratón de la enfermedad de Chagas crónica, hay disfunción endotelial, manifestada principalmente por cambios en la morfología de la arquitectura vascular y del miocardio, y la expresión de las moléculas de adhesión endotelial: e-selectina, VCAM-1, ICAM-1. Por otro lado, se determinaron los niveles séricos de TXA2 y PGE2 y se correlacionaron con la expresión de COX y sus isoformas COX1 y COX2.

Así, se observaron cambios histológicos indicativos de miocarditis crónica, principalmente por las características del infiltrado inflamatorio, asociado a engrosamiento de las paredes de arteriolas y entrecruzamiento y desorganización de las fibras miocárdicas, alteraciones evidentes a partir del día 24 después de la infección y que se mantiene luego de 90 días. La infección fue establecida con la inyección intraperitoneal de 500 tripomastigotes sanguíneos. El análisis inmuno-histoquímico de corazones infectados con *T. cruzi*, reveló expresión de moléculas de adhesión como ICAM, VCAM y e-selectina; además, aumentó el nivel circulante de la fracción soluble de ICAM (sICAM), que también es un marcador de daño endotelial.

Por otro lado, mediante estudios de *Western blot*, se pudo comprobar que en la cardiopatía chagásica la isoforma predominante es la ciclooxigenasa de tipo 2, tanto en modelos *in vivo* como *in vitro* de la infección. Además, esta actividad puede ser inhibida por la aspirina, lo cual es un hallazgo interesante, por cuanto se ha establecido que la aspirina inhibe predominantemente a la isoforma constitutiva.

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Daño y reparación del ADN en *Trypanosoma cruzi*, posible blanco terapéutico

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Trypanosoma cruzi, a parasitic protozoan, is the etiological agent of Chagas' disease, an endemic pathology in Latin America. The transmission of the disease is produced by an infected triatomine insect that upon feeding on mammalian blood, deposits feces with infective parasites (trypomastigotes) which enter the mammalian body mainly through the skin wound produced by the insect. Upon entering the body, the parasites invade macrophages taking a round, replicative form, the amastigote. After replication, the amastigotes transform back to trypomastigotes that invade heart, ganglia and other tissues. Drugs used for treatment of Chagas disease are mainly active in acute infection and present collateral effects.

Trypanosoma cruzi is exposed to ROS/NOS in its three cellular forms. Thus, in the intestine of the triatomine after a blood meal, Fe^{+2} is formed

and produces reactive species through the Fenton reaction affecting epimastigotes. On the other hand, trypomastigotes are under the attack of ROS/NOS in the parasitophore vesicle while amastigotes suffer the effect of the reactive species inside mammalian cells. To establish a chronic infection some parasites must resist the oxidative damage. Although all cellular macromolecules are subject to damage, the primary deleterious consequences of oxidative stress probably arise from damage to DNA. However, to date we are not aware about reports showing oxidative damage in *T. cruzi* DNA. Additionally, the capacity as well as the mechanisms responsible for the repair of the DNA damage is also unknown. We propose that the parasite DNA is damaged but it is repaired via the base excision repair pathway that is activated when *T. cruzi* is exposed to ROS/RNS, allowing its survival.

We report that H_2O_2 and NOO^- induce oxidative nuclear and kineplastid DNA damage in *T. cruzi* that may be partially repaired by the parasite. Furthermore, we show that both oxidative agents diminish cell viability of *T. cruzi* epimastigotes and trypomastigotes. This effect is significantly augmented in parasites subsequently incubated with the drug methoxyamine, an inhibitor of the apurinic/apyrimidinic enzymes involved in the base excision repair pathway of DNA repair, suggesting that the base excision repair pathway is indeed activated after oxidative damage to the parasite. The diminution of cell viability after ROS/NOS attack is most probably due to a population of parasites unable to repair their DNA. On the contrary, the maintenance of *T. cruzi* viability is a consequence of DNA repair mechanisms, most probably sustained by the base excision repair pathway.

We have cloned, expressed and identified by mass spectrometry three recombinant *T. cruzi* DNA repair enzymes (TcAP1, TcAP2 and NL1Tc). As analyzed by modelling, these enzymes present structural characteristics similar but not equal to mammalian ones. Using an antibody prepared against TcAP1 peptides we recognized presence of this enzyme in the three cellular forms of the parasite. Transfected TcAP1-GFP and TcAP2-GFP to epimastigotes show that TcAP1 and TcAP2 are localized in the nucleus but not in the kinetoplast of the parasite. Overexpression of both enzymes independently, increases survival of parasites when submitted to oxidative stress.

Our results show that *T. cruzi* DNA is damaged when exposed to H_2O_2 and NOO^- and that this damage is partially repaired by the parasite. Inhibition of the base excision repair pathway by methoxyamine diminishes parasite viability when exposed to oxidative agents. At least two *T. cruzi* enzymes of the base excision repair pathway were cloned and expressed. It is proposed that inhibition of DNA repair represents a possible therapeutic target for the control of *T.cruzi* infection, particularly if this target is articulated with the conventional drugs used for Chagas disease treatment.

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