

Monitoring of interactions between macrophage hybridoma and *Trypanosoma cruzi* by chemiluminescence.

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Intracellular parasitism of macrophages is a central point in the course of the Chagas' disease since these cells act alternatively as well as "host cells" than "killing cells" (1).

Considering the important role of macrophages in the immune response to *Trypanosoma cruzi*, it is of the highest interest to develop *in vitro* systems to test interactions macrophages /parasites.

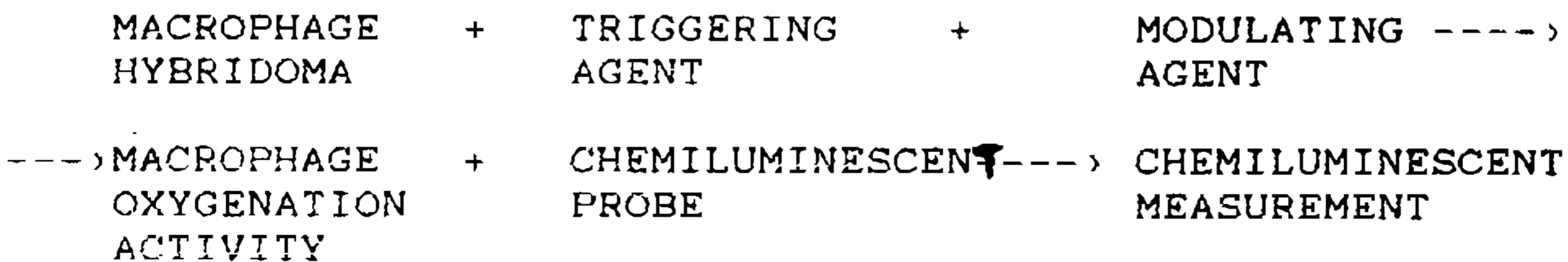
Most of the studies on macrophage/parasite relationships are conducted using peritoneal mouse macrophages. However, depending on their maturation stage, these cells are extremely heterogeneous (2).

Macrophage-like cell lines overcome only partially these withdrawals. Indeed, though homogeneous, such cells are not suitable for chemiluminescent monitoring, a sensitive assay to evaluate macrophage/parasite interactions.

Recently, new continuous macrophage cell lines have been developed by somatic hybridization between normal Balb/c mouse peritoneal macrophages and a 8- azaguanine resistant cell line (J-774-C2-E2-HAT) derived from J774-2 cell line (3).

These macrophage hybridoma keep a whole array of typical functions such as phagocytosis, tumoricidal and microbicidal activities, secretion of monokines and express Fc and C3b receptors. They are easy to grow, uniform and stable. When they are triggered (i.e with a lymphokine) interactions between macrophage membrane and the parasite (modulating agent) induce a signal easy to detect in a chemiluminescent assay:

CHEMILUMINESCENT ASSAY



Thus, these macrophage hybridoma constitute a new tool to test *T. cruzi*/macrophage interactions. Our results obtained with this model can be summarised as follow:

1) We present evidence of the intracellular multiplication of the parasite in macrophage hybridoma (optic and electron microscopy). Then, the percentage of infected macrophages and mean the number of amastigotes per infected cells are higher to values obtained with peritoneal mouse macrophages. This is probably due to the greater size of macrophage hybridoma.

2) We have measured the chemiluminescent signal induced by triggered macrophage hybridoma and incubated with *T. cruzi*, (ratio 20/1, 50/1 and 150/1 parasite per cell). This signal is proportionnal to the ratio parasite/macrophage. In addition, it is depending on the kind of triggering agent (T Cell Growth Factor, lipopolysaccharide, alone or in association). This indicates that parasite binding to the macrophage membrane induces a physiological modification and the emission of electrons from oxigen derivates resulting in a chemiluminescent signal.

3) Parasites were opsonised with various types of antibodies (anti-*T. cruzi* antibodies obtained by immunisation or infection). When added to triggered macrophage hybridoma, a more or less elevated chemiluminescent signal is recorded. These results suggest that antibodies and Fc receptors are implicated not only in the binding of parasite to the membrane but seem also to determine the level of the chemiluminescent response.

4) Fibronectin can modulate at least partially interactions between *T. cruzi* and peritoneal mouse macrophages (4). When parasites are incubated with human fibronectin then mixed to macrophage hybridoma, only a weak chemiluminescent signal is obtained. But when rabbit anti-fibronectin antibodies are added to "fibronectin opsonised" parasites, a strong signal is recorded. This is not the case with anti-fibronectin F(ab')₂ fragments. These results suggest that receptors implicated in the fibronectin parasite/macrophages binding do not stimulate the degradation of oxigen derivates.

These preliminary results indicate that interaction macrophage/parasite can be easily monitored and quantified by this new kind of *in vitro* model of infection and the

chemiluminescent assay.

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