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Frequency distribution of *Trypanosoma cruzi* in macrophages from resistant and susceptible strains of mice

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Summary. The frequency distribution of *Trypanosoma cruzi* inside macrophages from normal or chronically infected resistant and susceptible mice obeys a negative binomial type of distribution. This implies that an "aggregating mechanism" operates in *T. cruzi:* macrophage interaction.

Chagas' disease, a systemic disease, is caused by infection with *Trypanosoma cruzi*, an intracellular protozoan flagellate. It has been shown[3,4] that 2 different strains of mice, A/J (H-2^a) and C57 B1/10 (B10) (H-2^b) are susceptible and resistant to experimental *T. cruzi* infection. Moreover, these strains present different levels of intramacrophage parasitism after in vitro incubation of the parasites with resident peritoneal macrophages[4]. When peritoneal washout macrophages from normal A/J and B10 mice are incubated with trypomastigotes the time course curves for the percentage of infected macrophages are exactly the same. However, the accumulated means are higher in A/J than in B10 animals. When the washout macrophages are derived from chronically infected mice (mice at 60 days post-infection) both the percentage of infected macrophages and the accumulated means are significantly higher in the A/J than in the B10 mice (figure 1). Here we show that irrespective of the genetic profile or the stage of infection, the frequency distribution pattern of intracellular parasitism obeys a contagious distribution, mainly the negative binomial one. Resident peritoneal macrophages (Mø) from normal and chronic A/J and B10 mice were incubated in vitro with blood-form trypomastigotes of the Y strain. The number of intracellular parasites per cell, up to a total of 250 Mø, were counted at 1, 2, 8, 14 and 24 h of incubation. The frequency distribution curves were fitted to a negative binomial type of distribution[5]. The goodness-of-fit was based on the usual χ^2 statistic. The distribution curves of a typical experiment are shown in figure 2. In all the experimental situations, the distribution pattern fits a negative binomial type, except for the 24 h points where a best fit is found with a negative binomial truncated at the point $K=0$.

The contagious type of frequency distribution is very commonly found describing frequency distribution of living organisms in their natural habitat[6]. Natural populations tend to obey aggregated patterns of spatial distribution unless very improbable contingencies are taking place. True randomness would be very seldom found. A number of mathematical models comprising the compound Poisson series has been described as explanatory for those observed distributions[7], the negative binomial being one of them. The basic assumption is that a randomly distributed variable would have its Poisson lambda parameter varying over the entire field of observation.

Sample variances and means of the frequency distribution of *Trypanosoma cruzi* inside macrophages in normal and chronically infected A/J and B10 mice after different times of incubation

Time (h)	A/J				B10			
	Normal		Chronic		Normal		Chronic	
	s^2	$\bar{x}$	s^2	$\bar{x}$	s^2	$\bar{x}$	s^2	$\bar{x}$
1	0.8505	0.3360	0.3662	0.2080	0.4110	0.2160	0.1770	0.0880
2	1.8960	0.4600	1.7315	0.6160	0.4110	0.2160	0.2215	0.1240
8	7.7593	1.0880	14.7578	2.9280	2.0535	0.6440	4.3470	0.8800
14	4.9468	1.0320	10.5560	2.5160	0.8470	0.4200	5.7871	1.0640
24	3.8587	0.8560	9.3369	1.9800	1.6859	0.6040	0.4494	0.1280

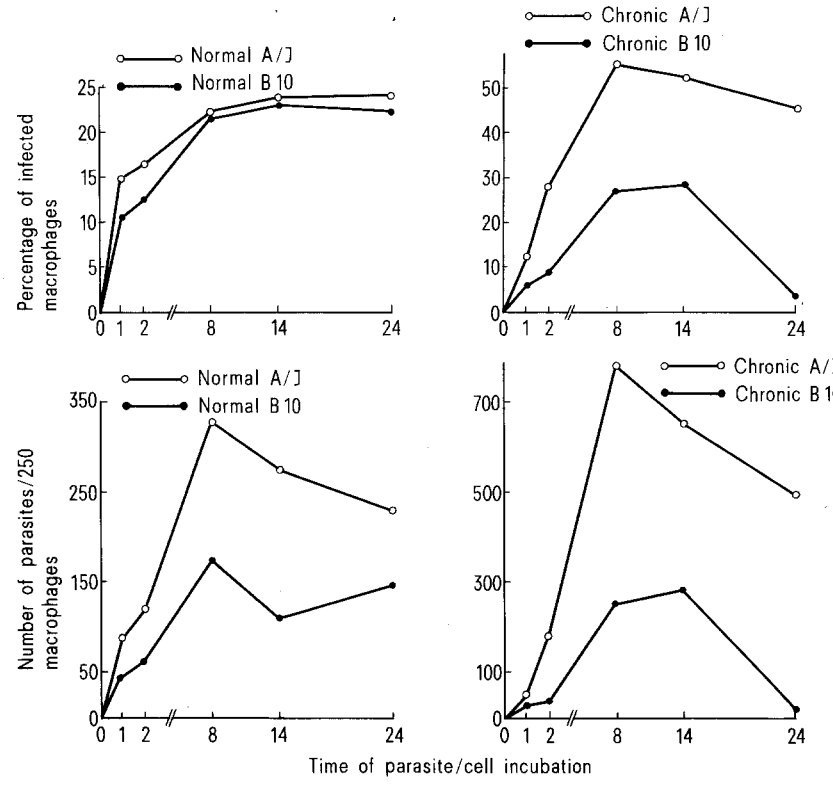

Fig. 1. Comparison of the in vitro infectivity of peritoneal macrophages from 2 inbred strains of mice by analyzing the percentage of infected macrophages and the total number of internalized parasites in a fixed number of cells.

In all the "contagious" models the striking feature is overdispersion, that is, a variance greater than the mean. The contagious distributions are in reality measuring the degree of clustering or aggregation of individuals from a population. Since parasitism can be described as an ecological relationship between 2 different populations, the host/parasite relationship can be quantified by the use of frequency distribution curves[8]. Of the several different reasons for which a negative binomial distribution might arise in a host/parasite, all of them have in common the aggregation tendency of the parasites. The aggregation unit or "quadrat" would of course range from a simple cell to an entire organism depending on the observation "distance". The sample variance (S^2) of our data is always greater than the sample mean ($\bar{x}$) providing sample dispersion coefficients ($s^2/\bar{x}$) higher than 1 (table 1). It should be noted that the overdispersion of the observed frequencies increases with the incubation time. We have used as a measure of aggregation the Ii index of dispersion described by David and Moore[9]. It is given by $s^2/\bar{x} - 1$. Randomness will make Ii tend to 0 while increasing aggregation increases Ii values. A correlation analysis between Ii and the accumulated mean of intracellular parasites gives positive correlation coefficients higher than 0.80. This means that the intracellular number of parasites increases exactly as the aggregation does. This fact would imply that, as time of incubation increases, more parasites are progressively found in some cells, while other cells remain non-penetrated. Since "contagious" distributions are due to "cooperativity" types of phenomena, in the sense that the occurrence of one event interferes with the chance of occurrence of a subsequent one, we are tempted to postulate that something of this order is occurring in the present situation. Since the maximum incubation time used in our experiments is insufficient for intracellular multiplication of the parasite[10] we can consider the number of intracellular forms as reflecting the number of internalized trypomastigotes (be it active penetration or phagocytosis). Actually the distribution pat-

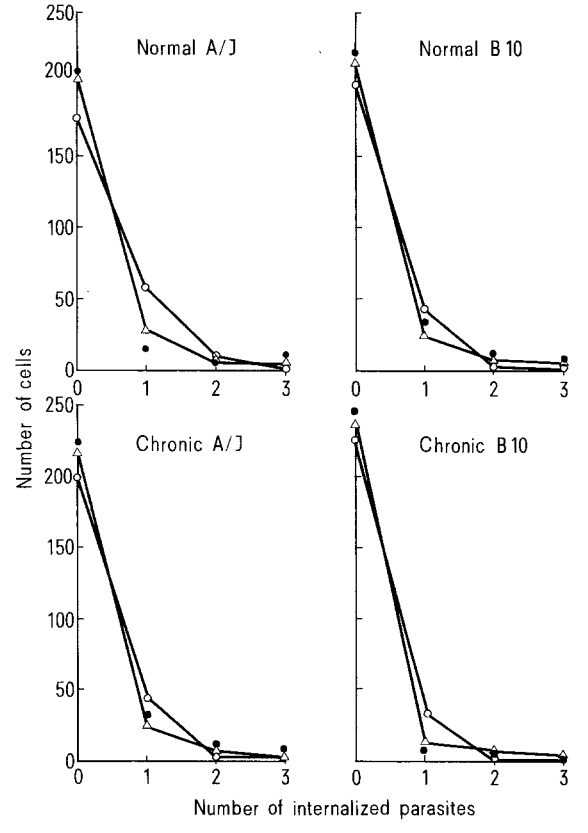

Fig. 2. Frequency distribution curves of parasites inside macrophages after 1 h of in vitro interaction. The calculated Poisson distribution (○——○) and the negative binomial distribution (△——△) are depicted in the graphs, together with the experimental points (●). The fit at all the hours studied is better with the negative binomial than it is with the Poisson distribution.

tern of intracellular parasites reflects the exact internalization pattern of trypomastigotes in Mø. Considering that the parasites are free to move and randomly establish contacts and interactions with all the available Møs one would expect a random distribution of intracellular forms, best described by a Poisson model. Since this is not the case, various mechanisms can be advanced to account for the intracellular aggregation. The first one would be a non-random killing of intracellular forms in the sense that cells with few parasites would be less able to lyse them than cells with a large number of forms. This does not seem to be the case, since at late incubation times, when the number of intracellular forms begins to fall, the degree of clustering also decreases. As a matter of fact, our data is a strong indication that intracellular killing occurs at random. Another possibility is that a clustered or contagious pattern of penetration occurs. One could suggest that once a Mø is penetrated by a parasite it becomes more susceptible to subsequent penetration, or that it "turns-on" resistance to subsequent penetration in adjacent nonpenetrated cells. The former possibility may be due to membrane alterations following penetration or even to some sort of chemotactic stimulus released by a penetrated cell. The induced resistance could also be due to the release of a cell factor, as indeed has been shown to occur after *T. cruzi* infection[11]. Our results tend to favor the "facilitating" hypothesis. Both normal resistant (B10) and susceptible (A/J) strains of mice present the same number of infected macrophages at a given moment throughout the incubation period (figure 1). This indicates that the initial sorting of cells by parasites, in the same number of Mø increases sharply in the A/J but not in the B10 mice. Also, one can see that new clusters arise at a similar rate in cells from both strains. If a protective soluble factor were to be released by Mø one would expect a decrease in the number of clusters with incubation time, in the B10 strain. Finally, a clustered frequency distribution could be due to the existence of a Mø sub-population with a different phagocytic capacity or penetrability. This last assumption could certainly be due to the existence of macrophages bearing special receptors for *T. cruzi*. As a matter of fact Nogueira and Cohn[12] have postulated the existence of a macrophage receptor for culture forms of *T. cruzi*. Hyde and Dvorak[13] observed a negative binomial frequency distribution of the Ernestina strain of *T. cruzi* in secondary bovine embryo skeletal muscle cells. We now show it to occur with a different strain of parasite in a cell populatin that is not only actively penetrated by parasites but also able to phagocytize them. The phenomenon also occurs in different strains of mice and irrespective of the total number of internalized parasites. This fact certainly means that this specific frequency distribution is measuring a very basic and general phenomenon underlying *T. cruzi*/host interaction.

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Dextranase-producing organisms in dental plaque from caries-free and caries-active naval recruits[1]

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Summary. Dental plaque samples from caries-free and caries-active naval recruits were assayed for the prevalence of dextranase-producing organisms. These organisms were found in the plaque of all of the subjects. Mean percentages of dextranase-producing organisms with respect to total colony count for the 2 groups of subjects were not significantly different.

The oral organism, *Streptococcus mutans*, produces extracellular glucans from sucrose which may promote plaque formation and dental caries by enabling masses of bacteria to adhere to teeth[2]. The glucans are composed of α-1, 6- and α-1, 3-linked glucosyl residues[3-5]. Certain plaque organisms elaborate dextranases which can hydrolyze the α-1, 6 linkages and thus may partially degrade the glucans[6,7]. If the dextranases from plaque organisms were to confer significant protection against dental caries through glucan degradation, a direct relationship between the prevalence of dextranase-producing organisms in plaque and caries resistance might be demonstrable. In this study we have compared the prevalence of these organisms in plaque samples from 2 groups of young men of widely differing caries experience.

Materials and methods. The subjects were 19 caries-free and 20 caries-active male US naval recruits, 17-25 years of age. Subjects designated caries-free had no evidence of active or past tooth decay, whereas each subject designated caries-active showed open lesions on at least 8 posterior teeth. Plaque samples were obtained on waxed dental floss by passing the floss between the contact points of the 1st and 2nd molar, and of the 1st molar and 2nd bicuspid of each dental quadrant. The 2 plaque samples from each quadrant were combined in thioglycolate-broth holding medium (minus carbohydrate and indicator, Difco Labs, Detroit, Mich.), and were sonicated. Additional details of these procedures have been described elsewhere[8]. The samples were diluted $1:10^3$ and $1:10^4$, and the dilutions were spread in duplicate onto heart infusion plates (Difco Labs) con-