INFLUENCE OF STATIC MAGNETIC FIELDS ON CELL VIABILITY, NECROSIS AND APOPTOSIS

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The static magnetic fields, in the range from 0 to 670 mT, heavily influenced not only the rates of viability, necrosis and apoptosis of human macrophages, but also the expression of their membranal protein markers CD14 and CD64. The infection with Mycobacterium tuberculosis led to a significant decrease of these harmful magnetic-field effects.

1. Introduction

This study was motivated by the fact that, while for three and half billion years life on Earth evolved in an environment where the static and oscillatory magnetic fields (MFs) were naturally combined, the progress of technology has dramatically changed their equilibrium during the last century. The new condition has caused alarm, since an unhealthy effect of the MFs, generated by 50-60 Hz high voltage power transmission lines, video display terminals, electric blankets and other home appliances, has been revealed (1-5). The belief that the field intensity, in the case of these sources, is rationally fixed below a risk threshold is seen as an objection to the alarm. However, the initial information was contradictory: on the one hand, a benefit from the low-energy pulsed MFs in non-union bone fracture healing and in cell regeneration was reported (6); on the other, the influence on nerves of an MF at 50-60 Hz caused concern (7). Some data suggested that, although without benefit, no direct tumorigenic or mutagenic effects could be attributed to the MFs, since they did not

cause DNA damage and did not interfere with the rate of DNA break formation due to clastogenic treatments (8). Instead, a number of data proved to be in favour of the second possibility: although the mechanism explaining putative co-carcinogenic or co-mutagenic MF effects was thought to be nongenetic, the MFs were claimed to enhance the mutation rates of cells exposed to mutagenes (9), increase the tumor cell survival after various cytocidal therapies (10) and increase the tumor rate in cancer-prone mice strains (11). Also, the MFs were claimed to alter the rate of transcription (12), influence the penetration of Ca^{2+} from the extracellular environment into the cell (13) and decrease the rate of spontaneous cell death (14). Moreover, an MF of 2 mT at 50 Hz was found to influence the Xenopus laevis metamorphosis: in the course of a 65-day exposure to the field, while the survival of the tadpoles showed a notable decrease, a parallel 6-day shift in their maturation frequency and a parallel impairment of their metamorphosis were observed; the metamorphosis was successful for 85% of individuals in the unirradiated tadpole population and for 45% of individuals in the irradiated tadpole population (15).

An investigation was undertaken by us to compare the behaviour of Friend erythroleukemia (FL) cells in a solenoid (SLD), where the MF was 70 μT at 50 Hz plus 45 μT DC of the Earth, with that of the same cells in a magnetically shielded room (MSR), where the MF was attenuated to 20 nT DC and 2.5 pT AC (the control laboratory MF corresponded to 45 μT DC and a stray 50 Hz field below 0.2 μT). Hence, a first set of experiments (14) showed that the culture growth cycle (CGC) of cells maintained inside the SLD was slightly accelerated compared to that of cells maintained outside the SLD. This stimulation probably depended upon the sensitivity of the cell cycle to an MF, because, inside the SLD, the percentage of G_1 cells slightly increased during the CGC, whereas that of S cells slightly decreased. Acceleration of growth was detected soon after exposure of the cultures to the SLD field, and growth did not change further if the action of the field continued for a long time, accounting for adaptation. The SLD field also caused a small increase of cell survival without

influencing cell volume. By contrast, the CGC of cells maintained inside the MSR was slightly decelerated compared to that of cells maintained outside the room. The essential absence of any field inside the MSR caused, in addition, a small increase of cell volume, whereas, during the CGC, the percentage of G_1 cells decreased and that of S cells increased. In another set of experiments (16), the generalized polarization function of the fluorescent probe 2-dimethylamino-6-lauroylnaphtalene was used to evaluate the lipid dynamics in FL cell membrane, with and without MFs. The control values of this function varied during the CGC, showing decreased lipid dynamics 24-48 hrs from the cell seeding. When the cycle occurred in the SLD (short-term 4-day exposure), the membrane lipid dynamics decreased by about 10% during the same time period. After long-term (184 days) or extremely long-term (395 days) exposure of the cells to the MF, little additional variation in the membrane lipid dynamics was observed, again suggesting the occurrence of an adaptation. Additional observations (17) showed that, in the range from 0.6 to 66 mT DC, the static MFs were able to decrease the rate of apoptosis induced by several agents in various types of human cells. This was not due to a change in the mode of cell death (i.e. to necrosis) or to a delay of the process itself; rather, the presence of static MFs allowed the indefinite survival and replication of the cell hit by apoptogenic agents. The protective effect was found to be mediated by the ability of the fields to enhance the Ca^{2+} influx from the extracellular medium; accordingly, it was limited to those cell systems where the Ca^{2+} influx had an antiapoptotic effect. Thus, the static MFs might interfere with human health by altering/restoring the equilibrium between the cell death and the cell proliferation. This set of experiments led us to conclude that the rescue of damaged cells might be the mechanism explaining why MFs, which are not mutagenic "per se", are often able to increase mutation and tumor frequencies, although in certain instances an adaptation to the MFs would occur. In other words, this would explain why life is still possible in the modern environment. This contribution concerns the influence of static MFs on the human monocyte-derived macrophages (MDMs), comparing their behaviour before

and after the infection with Mycobacterium tuberculosis (MTB). In addition to the fact that the field dramatically changed the number of bacteria present in a macrophage, the insertion of bacteria into macrophage phagosomes and the bacterial division itself in the macrophage cytosol, it shows that the MDMs, when infected with the MTB, became able to recover some viability that was previously compromised by the static MFs. This unexpected phenomenon suggested that the so-called newly 'emerging diseases', including tuberculosis, should be considered not only in the framework of studies on stress or on smog, but also in the context of nonionizing MF pollution.

2. The CD14 and CD64 macrophage markers showed a similar magnetic-field dependence and a partially different response to Mycobacterium tuberculosis

The collected information regarding the CD14 (18) and CD64 (19) markers served for a correct MDM identification. It allowed a background analysis of the MDM response to the investigated static MF intensities, in the absence and in the presence of MTB infection. In the case of the uninfected MDMs, there appeared a qualitatively similar biphasic MF dependence of both the markers: while the CD14 expression dropped by about 20%, at 0 to 232 mT (fig. 1a), in the same range of MF intensities the CD64 expression dropped by about 40% (fig. 1c); while the CD14 expression returned to its initial value, at 670 mT (fig. 1b), at the same MF intensity the CD64 expression again increased but did not reach its initial value (fig. 1d). The fact that at 670 mT both the markers tended to recover their initial expression accounted for an MDM adaptation, in contrast to the harmful effect of the MFs at 0 to 232 mT. In the case of the MTB-infected MDMs, all values of the CD14 marker ($P < 0.02$) and particularly all those of the CD64 marker ($P < 0.03$) showed a general decrease at 0 to 670 mT, compared to the values characterizing the uninfected MDMs. However, while at 670 mT the CD14 percentage tended to recover its full initial value, accounting for an adaptation phenomenon (fig. 1 a,b), at this same higher MF intensity the CD64 percentage tended

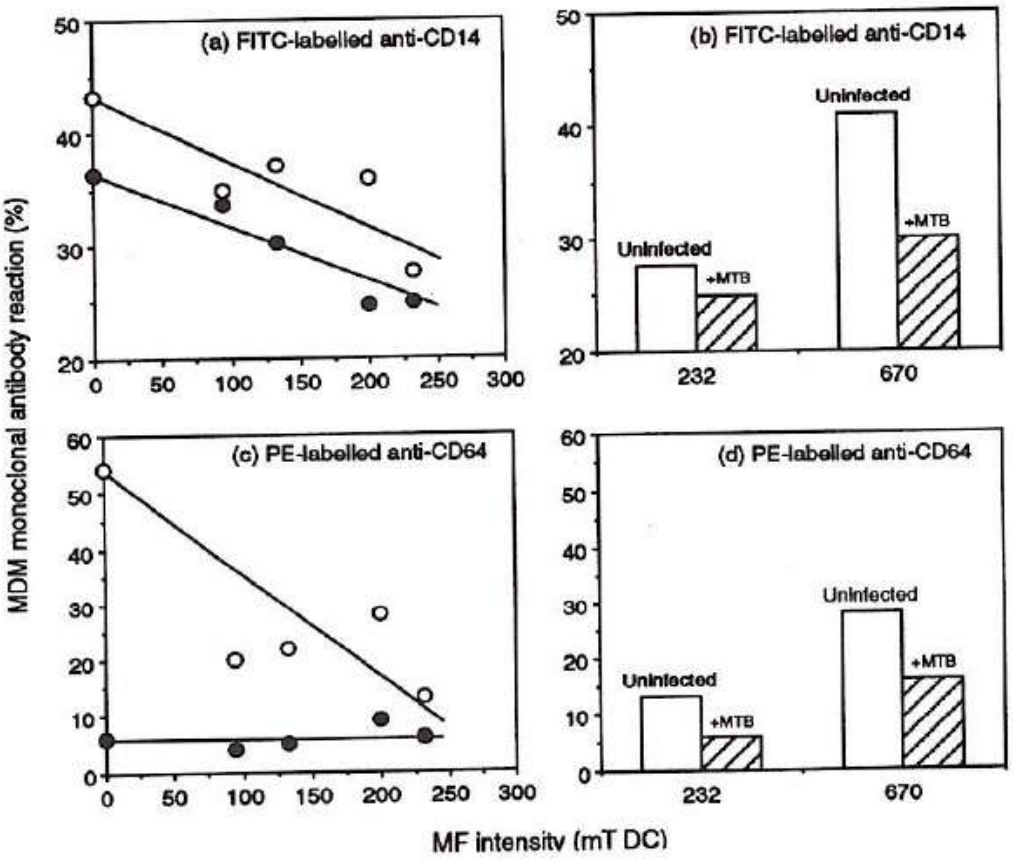

Fig. 1 - The CD14 and CD64 macrophage labelling developed as a function of the static magnetic field intensity. An appropriate sample of macrophages, separated from the PBMCs attached in 1 hr to the bottom of a flask, was subdivided into a number of equal samples each consisting of $1 \cdot 10^6$ MDMs in 1.5 ml of RPMI-1640 medium. One of these samples was quickly tested cytofluorimetrically to verify the CD14 and CD64 expression in the MDMs. Other five MDM samples were maintained for 5 days on the magnetic disks, while a control MDM sample was kept in the absence of any MF. At the end of the fifth day of exposure to the static MFs, each sample (including the control) was washed twice with PBS, while the MDMs were detached from the dish and subdivided into three parts: the first part served as blank; the second served for detection of the CD14 marker (a,b); the third served for detection of the CD64 marker (c,d). A parallel set of equal MDM samples, after a 5-day permanence in culture without MF, was infected with the MTB and maintained for other 2 days on the magnetic disks (a control MTB-infected MDM sample was kept for 2 days in the absence of any MF). At the end of the second day of exposure to the static MFs, each MTB-infected MDM sample was washed twice with PBS, detached from the dish and subdivided in three parts to determine, as before, the blank and the CD14 (a,b) and CD64 (c,d) markers. (O and white columns) Uninfected MDMs; (● and black columns) MTB-infected MDMs. The points represent the mean of three experiments showing, for (a), a $P < 0.02$ and, for (b), a $P < 0.03$.

to a further increase, accounting for a stimulation phenomenon (fig. 1 b,d). This could be judged in relation to the fact that, at 0 to 232 mT, while in the case of the uninfected MDMs the CD14 expression sharply dropped (fig. 1a), in that of the MTB-infected MDMs the CD64 expression essentially remained at a minimum (fig. 1c). Such a difference suggested that the same MF-sensitivity, in turn, could be on the basis of the different role played in the MTB-infected MDMs by the CD14 vs. the CD64 functions. It should be remembered that, at MF zero, the values of both the marker percentages (like those of the MDM viability, necrosis and apoptosis, shown below) did not coincide in the uninfected and MTB-infected MDMs, since the former remained on the magnetic disks for 5 days, from the inoculum, while the latter remained on them during the next 2 days.

3. The static magnetic fields almost fully depresses the viability of the uninfected macrophages and only partially that of the Mycobacterium tuberculosis-infected macrophages

In the uninfected MDMs, the almost similar MF-dependent biphasic patterns of the CD14 and CD64 functions (fig. 1 a-d) corresponded fairly well to a biphasic pattern of the MF-dependent MDM viability. It dropped by about 60%, at 0 to 232 mT (fig. 2a), and remained unchanged, at the level of 10%, at 670 mT (fig. 2b). In distinction to the influence exerted on the CD14 and CD64 functions by the MF at 670 mT DC, in the uninfected MDMs, there was no adaptation of viability to this field. There was, rather, a survival of an MF-resistant MDM fraction. An almost full adaptation of viability to 670 mT took place, on the other hand, in the case of the MTB-infected MDMs (fig. 2a,b): when infected with the MTB, the MDMs not only recovered a part of their MF-inhibited viability, at 0 to 232 mT DC, but recovered it totally, at 670 mT ($P < 0.004$). In terms of percentages, the depression of viability caused by the static MFs in the MTB-infected MDMs was much less, compared to that affecting the uninfected MDMs: while at 0 to 232 mT the viability of uninfected MDMs dramatically dropped by about 60%, as mentioned, in the same range of

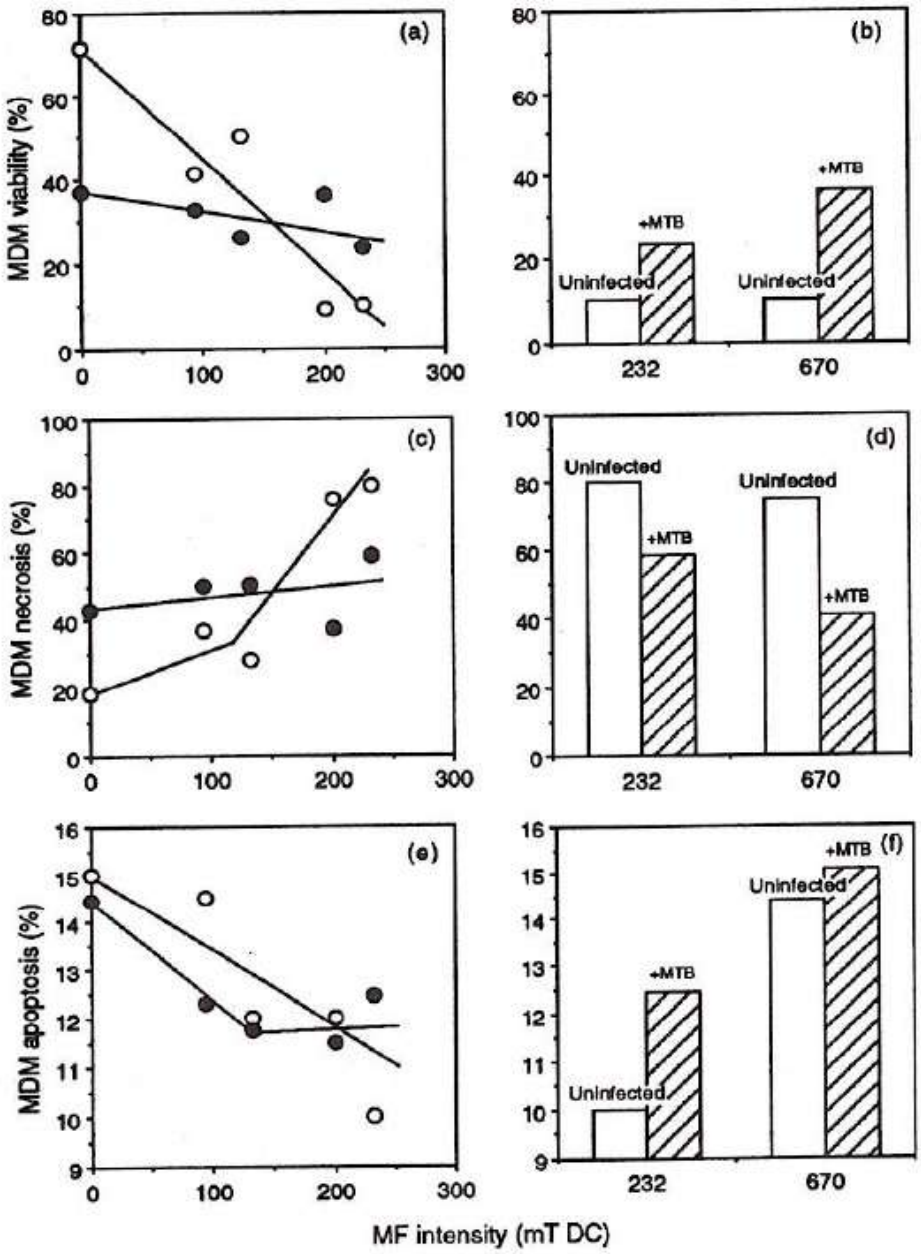

Fig. 2 - The macrophage viability, necrosis and apoptosis developed as a function of the static magnetic field intensity. An appropriate stock of MDMs, separated from PBM cells attached for 1 hr to the bottom of a flask, was subdivided (as for fig. 1) into a number of equal samples each consisting of $1 \cdot 10^6$ MDMs in 1.5 ml of RPMI-1640 medium. Five MDM samples were maintained for 5 days on the magnetic disks, while a control MDM sample was kept in the absence of any MF. At the end of the fifth day of exposure to the static MFs, each sample was washed twice with PBS, while the MDMs were detached from the dish, treated with PI and analyzed cytofluorimetrically. A parallel set of samples (each consisting as well of $1 \cdot 10^6$ MDMs in 1.5 ml of RPMI-1640 medium), after a 5-day permanence in culture without MF, was infected with the MTB and maintained for other 2 days on the magnetic disks (a control MTB-infected MDM sample was kept for 2 days in the absence of any MF). At the end of the second day

of exposure to the static MFs, each MTB-infected MDM sample was washed twice with PBS, detached from the dish, treated with PI and analyzed cytofluorimetrically. (a,b) Viability of the uninfected MDMs vs. viability of the MTB-infected MDMs ($P < 0.004$); (c,d) necrosis of the uninfected MDMs vs. necrosis of the MTB-infected MDMs ($P < 0.01$); (e,f) apoptosis of the uninfected MDMs vs. apoptosis of the MTB-infected MDMs ($P < 0.05$). (O and white columns) Uninfected MDMs; (● and black columns) MTB-infected MDMs. The values represent the mean of three experiments.

MF intensities that of the MTB-infected MDMs dropped by about 15% (fig. 2a). Thus, the MF effect on the MDMs was softer. In this weaker influence, one could see one of the possible reasons for the almost complete recovery of the MDM viability at 670 mT.

4. The static magnetic fields induced the highest degree of necrosis in the uninfected macrophages and only partially in that of the Mycobacterium tuberculosis-infected macrophages

As expected from the comparison with the MF dependence of the MDM viability (fig. 2a,b), the MDM necrosis vice versa increased by about 60% at 0 to 232 mT (fig. 2c) and remained unchanged at the level of about 80% at 670 mT (fig. 2d). Such a higher MF irradiation did not cause a complete extinction of the MDM population. This accounted either for the occurrence of an adaptation phenomenon or for the selection of an MF-resistant MDM fraction. However, the fact that in the MTB-infected MDMs at 670 mT the percentage of necrosis completely returned to its initial value was in favour of the first possibility. The observation that in general the rate of necrosis was inhibited by the MTB infection (fig. 2c,d) was also of importance: it increased by about 10% at 0 to 232 mT only ($P < 0.01$). This could suggest that at 670 mT the MDMs had an easier chance to avoid necrosis. In other words, the MTB infection would depress the MDM sensitivity to the harmful MF effect.

5. The static magnetic fields inhibited the apoptosis in the uninfected macrophages much more than in the Mycobacterium tuberculosis-infected

macrophages

The pattern of the MDM apoptosis (fig. 2e,f), from the standpoint of formal resemblance, was partially similar to that of the MDM viability (fig. 2a,b): it went down, at 0 to 232 mT, and returned to its initial value at 670 mT (at this higher MF intensity the MDM viability was stabilized at its minimal value). Let us analyze separately the two parts of the MF-dependent MDM apoptotic development. In its first part, there was an intrinsic divergence, with respect to the MDM viability: while at 0 to 232 mT the MFs were harmful for the MDM viability (fig. 2a), at the same intensities the MFs caused a decrease of the MDM apoptotic rate (fig. 2e). This accounted for a decreased capacity of the MDMs for self-destruction, under the static MF effect, in agreement with our previous finding showing that the static MFs' ability to modulate the Ca^{2+} fluxes causes a reduction of the rate of the stress-induced apoptosis (17). Thus, at 0 to 232 mT, with respect to the capacity for self-destruction by the MF-stressed MDMs, the cell response would leave an alternative: while a large portion of MDMs underwent necrosis (fig. 2c), a small portion of them tended to survive by inhibiting their own programmed death. In the second part of the MF-dependent MDM apoptotic development, the situation was inverted: at 670 mT the apoptotic rate returned to its initial value (fig. 2f). Thus, the higher MF intensity re-established the capacity of the MDMs for self-destruction, as it was before the MF application: a large portion of the MDMs continued to die by necrosis at 670 mT (fig. 2d), while a small portion of them conserved the ability to die by apoptosis (fig. 2f). So, in contrast to the directly harmful effect of the MFs at 0 to 232 mT, there occurred a variation of the MDM sensitivity to 670 mT in the sense that the MDMs re-established their capacity for self-destruction. In the case of the MTB-infected MDMs, the MF-dependent apoptotic development essentially did not change (fig. 2e,f). With respect to the uninfected MDMs, there was just a quantitative difference, namely a reduction of inhibition of the MDM apoptosis caused by the static MFs ($P < 0.05$). Such a reduction should be interpreted in the sense that, while at 0 to 232 mT the MTB infection caused the maintenance of

'surviving' MDMs by apoptosis (fig. 2e), at 670 mT the situation returned as it was in the case of the uninfected MDMs (fig. 2f).

6. Concluding remarks

The main aim of this investigation was to verify whether certain artificial MFs of given intensities are really harmful to living matter, since this possibility was so largely debated in the scientific community (1-5,20). The interest depended not only upon the theoretical question concerning the reason for the origin and evolution of life in the Earth's MFs (21), but also upon the practical question concerning the efficiency of concrete biological functions in static MFs of relatively high intensities (22,23). In harmony with the background knowledge suggesting the influence of low-frequency/low-intensity MFs on the transport of Ca^{2+} ions across the membranes (24,25), our previous work showed the inhibition of programmed cell death exerted by static MFs, exactly via a Ca^{2+} influx (17). In that case, the MF intensities varied from 0 to 66 mT. The average of these values overcame the MF intensity of the Earth by almost 1,000 times, oscillating around 45 μT (14,16). In the present work, compared with the geomagnetic field, the MF intensities were increased further by about 10,000 times, namely from 0 to 670 mT. The use of these MF intensities was mainly motivated by two reasons. On the one hand, it was due to the peculiarities of the investigations in Biophysics of Space dealing with the homeostasis of life inside or outside a closed cabin, since some sky-blue bodies do not originate magnetism while others irradiate strong MFs (21). On the other hand, it was due to the fact that many workers, including scientists involved in NMR or EPR experiments, for example, cannot fully avoid the risk of being exposed to static MFs of high intensities (26). The choice of the MDMs as target cells for the static MF action was motivated by the supposition that any stress signal should interfere first of all with the cells of the immune defence system. There existed, in fact, information regarding the MF-dependence of crucial immune reactions (27,28). Hence, the static MF effect on the MDMs, estimated on the basis of the percentage of their viability vs. that of their

necrosis or apoptosis, proved to be always dangerous from 0 to 670 mT, although there was an intriguing difference in the cell response before and after 232 mT. Before this critical MF intensity, the patterns of viability (fig. 2a,b), apoptosis (fig. 2e,f) and CD14 (fig. 1a,b) and CD64 (fig. 1c,d) expression opposed the increasing pattern of necrosis (fig. 2c,d) as in a specular reflection. After 232 mT, the MDM behaviour changed dramatically. While the patterns of viability and necrosis remained at a plateau, with the first at a lowest level and the second at a highest level (fig. 2b,d), there was a parallel increase in the development of apoptosis (fig. 2f) and expression of the CD14 and CD64 markers (fig. 1b,d). For the sake of clarity, while before 232 mT the irradiated MDMs mostly died by necrosis, after this MF intensity the irradiated MDMs mostly died by apoptosis. Of particular interest was the fact that, from 232 to 670 mT, the CD14 and CD64 expression no longer reflected the development of viability: it went up, conflicting with patterns of viability (maintained at the minimum) and necrosis (maintained at the maximum). Probably, the apoptotic state could facilitate the detection of the two membranal proteins: the ability of the apoptotic MDMs to express them highly - an event not yet described for apoptosis (29,30) or CD14 and CD64 (31,32) - could be caused, for instance, by the MF-dependence of the membranal fluidity (16). The parallel increase of the two markers and apoptosis could also account for an adaptation phenomenon, as in the case of the same membranal fluidity (16). However, in the present case, such a possibility conflicted with the fact that after 232 mT the MDMs continued to die because of the increasing apoptotic rate. Another notable fact was the divergence in the MDM death by necrosis or apoptosis before 232 mT: while the necrosis increased, the apoptosis decreased, confirming previous observation (17). Such a countercurrent response would imply an attempt of the MDMs to defend themselves against the inevitable harmful MF effect. This suggests that any further increase of the MF intensity, from 232 to 670 mT, would overcome this extreme cell defence too.

The second aim of this study was to ascertain any eventual influence of

the mycobacterial infection (33,34) on the response of the MDMs to static MFs. So, figures 1 and 2 revealed that the considered MDM patterns, although flattened, roughly developed in the same way as those observed in the absence of MTB infection, with a common critical point at 232 mT. A closer look at the results showed, in fact, that the situation essentially changed after this MF intensity only: (i) at 670 mT, the MDM viability was not maintained at the minimal level (as in the absence of MTB infection), but tended to increase; (ii) at 670 mT, correspondingly, the MDM necrosis was not maintained at the maximal level (as in the absence of MTB infection), but tended to decrease again; (iii) at the same MF intensity, the MDM apoptosis (as in the absence of MTB infection) increased in parallel with the increase of the two membranal protein markers (however, since from 0 to 232 mT the MTB infection strikingly inhibited the CD14, its resumption at 670 mT again favoured the idea of an adaptation phenomenon). Thus, under the effect of the MTB infection, taken as a whole, the MDM viability, necrosis and apoptosis at 670 mT tended to return to their initial values, after a simultaneous decrease at 232 mT (among the various parameters, not considering the common flattening of the corresponding curves due to the MTB infection itself, the one which essentially did not change - without or with MTB - was the apoptotic rate). In other words, while from 0 to 232 mT the MTB infection attenuated the decrease of the MDM viability vs. the increase of the MDM necrosis, from 232 to 670 mT the MTB infection caused a recovery of MDM viability and a corresponding decrease of MDM necrosis. Consequently, while the two membranal protein markers appeared to distinguish the apoptotic MDMs especially, the MTB infection was clearly able to combat at a certain extent the harmful effect of the intensive static MFs on the MDM viability. This led further to the essential matter of the present investigation, suggesting that the human MDMs, when infected with the MDMs, partially recovered viability inhibited by strong static MFs.

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