Admixture of intact or lysed platelets to lymphocyte cultures results in higher chromosome aberration frequencies

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We have examined the effect on chromosome aberration frequency of in vitro admixture of intact platelets or platelet lysates to lymphocyte cultures. A roughly linear correlation was found between platelet concentration in the culture medium and the level of breaks and gaps, with higher platelet concentrations corresponding to higher aberration frequencies. We draw the conclusion that a platelet-derived factor interferes with the DNA breakage-repair mechanism of dividing lymphocytes. Awareness of the importance of in vitro platelet concentrations is necessary in cytogenetic studies evaluating genotoxic effects of chemical substances or constitutionally determined breakage-repair differences.

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The frequency of structural chromosome aberrations (gaps, breaks and exchanges) in lymphocyte cultures is known to vary considerably, not only between different individuals and laboratories, but also for the same person investigated on different occasions (BROGGER et al. 1984; SINHA et al. 1986). Although in vivo conditions, for example acute virus infections, undoubtedly may have considerable effects on recorded breakage frequencies, a major part of the observed variability may be due to in vitro factors. The serum is the least well defined component of the culture medium, and variation in serum concentration has been demonstrated to influence the aberration frequency (JOHANSSON and MERTENS 1986).

The effect of serum on chromosome fragility could conceivably be due to a number of parameters. We have in the present study examined the effect on chromosome aberration frequencies of platelets, intact or lysed, in the culture medium.

Material and methods

Peripheral blood samples from healthy adults were collected in 10 ml heparinized vacutainers. Ten drops from each sample were cultured at 37°C for 72 hours in 10 ml McCoy's 5 A medium supplemented with 20 % fetal calf serum, glutamine, antibiotics, and phytohaemagglutinin. Colcemid (0.1 μg/ml) was added during the last 30 min, whereupon the cultures were exposed to hypotonic shock in 0.075 M KCl for 15 min followed by fixation in three changes of methanol: acetic acid (3:1). The chromosomes were spread on clean pre-cooled wet slides and stained in a 5 % Giemsa-buffer solution.

The platelets were obtained from platelet concentrate provided by the Central Blood Bank of the University Hospital. The concentrate was prepared from healthy donors as described by HOGMAN et al. (1983), and contained approximately 10⁹ platelets/ml. Part of it was frozen quickly to -70°C for one hour and then thawed at 37°C in a water-bath to prepare the lysate.

The lymphocyte cultures were exposed to different concentrations of intact and lysed platelets according to the following protocols:

Experiment 1: Five cultures were initiated from each of four parallel blood samples. Four were exposed to 0.1 ml, 0.2 ml, 0.3 ml, and 0.4 ml of the platelet concentrate corresponding to 10⁷, 2×10⁷, 3×10⁷, and 4×10⁷ platelets per ml of culture, respectively. The fifth culture was left untreated as a control.

Experiment 2: Four cultures were initiated from each of two parallel blood samples. To three of the
cultures was added 0.1 ml, 0.2 ml, and 0.3 ml platelet lysate, corresponding to \(10^7\), \(2 \times 10^7\), and \(3 \times 10^7\) lysed platelets per ml. The fourth culture was used as control.

All preparations were coded and the cytogenetic analyses performed in a blind manner. The following aberrations were accepted in accordance with the recommendations laid down by ISCN (1985): Chromatid gaps (ctg), chromosome gaps (csg), chromatid breaks (ctb), chromosome breaks (csb), acentric fragments (ace), chromatid exchanges (cte), dicentrics (dic), markers (mar), and rings (r). One hundred mitoses of good quality were analyzed from 1 to 4 slides from both treatment and control preparations. The mean of the total chromosomal aberrations was computed for each treatment level and for the control culture in both experiments. The effect of different platelet concentrations was studied by an analysis of covariance allowing for the intraindividual variation.

**Results**

The findings of experiment 1 are given in Table 1; those of experiment 2 in Table 2. As can be seen, chromatid breaks were the most frequently observed aberrations in both experiments, followed by chromosome breaks. The numbers of aberrant cells in the two experiments are graphically displayed in Fig. 1 and 2. The increased aberration rates appear to be dose-dependent. Thus, there was a statistically significant effect of increasing platelet concentrations in both experiments (\(P=0.019\) and \(P=0.016\), respectively; two-sided tests).

**Discussion**

We have demonstrated that admixture of intact or lysed platelets leads to higher levels of gaps and breaks in lymphocyte cultures. The amount of platelet-derived factors is unknown in commercially available serum used in tissue cultures, but may contribute to the higher level of aberrations seen with very high serum concentrations (JOHANSSON and MERTENS 1986). It is likely that variability in platelet lysate concentration may also contribute to the differences in aberration frequencies often experienced with different serum batches. Our data emphasize that this parameter must be kept constant in all investigations comparing the genotoxic effects of chemicals and in studies of genetically determined
### Table 1. Chromosome aberration levels in lymphocyte cultures in response to admixture of intact platelets

| Platelets/ml | Mean frequency of chromosome aberrations (%) | ctg | csg | ctb | csb | ace | dic | mar | Total |
|--------------|---------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-------|
| 0            |                                             | 3.25| 0.25| 4.75| 3.0 | 0.25| -   | -   | 11.5  |
| $10^7$       |                                             | 3.5 | 2.25| 5.25| 3.75| 2.25| 0.25| 0.25| 17.5  |
| $2 \times 10^7$ |                                           | 4.5 | 4.5 | 9.0 | 5.25| 1.75| -   | -   | 25.0  |
| $3 \times 10^7$ |                                           | 6.25| 1.5 | 6.5 | 4.0 | 0.75| -   | 0.25| 19.25 |
| $4 \times 10^7$ |                                           | 5.25| 2.25| 7.5 | 6.25| 0.25| 0.25| -   | 21.75 |

### Table 2. Chromosome aberration levels in lymphocyte cultures in response to admixture of platelet lysates

| Platelets/ml | Mean frequency of chromosome aberrations (%) | ctg | csg | ctb | csb | ace | cte | Total |
|--------------|---------------------------------------------|-----|-----|-----|-----|-----|-----|-------|
| 0            |                                             | 0.5 | 0.5 | 3.0 | 2.5 | -   | -   | 6.5   |
| $10^7$       |                                             | 0.5 | 1.5 | 7.5 | 6.0 | 1.0 | -   | 16.5  |
| $2 \times 10^7$ |                                           | 0.5 | 1.0 | 10.5| 7.5 | 0.5 | -   | 20.0  |
| $3 \times 10^7$ |                                           | 2.0 | -   | 12.0| 3.5 | 3.0 | 1.0 | 21.5  |

Chromosome fragility. The possible effects on chromosome breakage frequency of variable platelet levels in vivo remain to be elucidated.

Additional credence is lent to our findings by the demonstration that a platelet-derived factor inhibits unscheduled DNA synthesis (UDS) in resting lymphocytes (PERO and VOPAT 1981). It is likely that this reduction in UDS reflects suppressed DNA repair. The evidence for a DNA-damaging effect of presently unknown platelet factors therefore stems from both cytogenetic and biochemical data.

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