

APPENDIX H:

DETECTION AND SIGNIFICANCE OF GENETIC ABNORMALITIES

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Cytogenetic tests

Cytogenetic abnormalities can be detected through two principal approaches, the use of molecular techniques to study gene sequences at the level of individual genes and the detection of changes at the chromosomal level. The latter approach was used for testing in the SAS soldiers. Three methods of detecting chromosomal abnormalities and the limitations of these tests are described below.

i) *Chromosome Aberrations*

The study of chromosomal aberrations in peripheral blood lymphocyte cultures has been used routinely during the last three decades to survey exposure of humans to various genotoxic agents. There are two types of chromosome aberrations; asymmetrical unstable aberrations (eg dicentric) and symmetrical stable aberrations (eg translocations) (Tucker et al 1996, 1997). Dicentric and other types of unstable aberrations are lethal when cells undergo division, and are therefore quickly eliminated from proliferating cell compartments such as bone marrow. Hence unstable aberrations in lymphocytes are useful as transient biomarkers of recent exposure (Tucker et al 1997) but the test needs to be performed soon after exposure has occurred. Stable new chromosome rearrangements such as translocations are passed onto daughter cells during cell proliferation and so persist throughout a lifetime and broadly reflect an individual's cumulative exposure to clastogens (Tucker et al 1997). Many cells need to be evaluated to obtain statistically significant results in comparison to controls.

iii) *Sister Chromatid Exchange (SCE)*

A chromatid exchange reflects a chromosome break followed by an incorrect rejoining as part of the chromosome repair mechanism. This technique has been used for monitoring chemical exposure for two reasons, it is a sensitive indicator of damage having occurred and it is relatively easy to learn and interpret. In an individual with a genetic disorder of chromosome repair, up to 80-90% of the chromosomes show chromatid exchange per cell. In contrast a normal person will have on average only 7% SCEs per cell. The increased frequency of SCE *in cells* would either indicate an inherited genetic tendency or that some clastogenic event has happened in the past. However, there is limited understanding of the mechanism underlying the formation of SCE (Tucker et al 1997). The use of SCE has been criticised because significant increases have been obtained from non-genotoxic compounds such as NaCl and KCl (Tucker et al 1997). SCE evaluated following in-vivo exposure have shown limited persistence and accumulation, which appear to reflect DNA repair processes as well as cell turnover (Tucker et al 1997).

iv) *Micronucleus test.*

Many studies on the effect of chemical exposure have looked at stained white cells for micronuclei in the cytoplasm as a large number of cells can be counted quickly. A micronucleus is the secondary effect of a chromosome that has broken and reformed with the broken ends fusing to form a ring. The rings interlock as the chromosomes duplicate during cell division and as the cells

separate into two the ring is pulled out into the cytoplasm forming a micronucleus. Micronuclei can also form as a result of spindle disruption (aneuploidogenesis) (Tucker et al 1997).

Studies in the chemically exposed show that this is quite a reproducible method of looking at DNA damage. However, other factors known to influence results are increasing age, gender and smoking as well as the degree of exposure to chemicals, plasma B12 and plasma homocysteine levels. There is also some assessment bias and culture media effects in the scoring of micronuclei. The micronucleus test is only useful when conducted within a short time after exposure (Tucker et al 1996).

Studies of structural chromosome abnormalities in peripheral blood lymphocytes for cancer risk assessment

The identification of structural chromosomal aberrations in cancer tissue has prompted work on a surrogate for use in cancer risk assessment: structural chromosomal aberrations in peripheral blood lymphocytes. Four cohorts that used prospective data were identified (ie the cytogenetic examination of peripheral blood lymphocytes was performed before the cancer occurred). These cohorts were from Italy (Bonassi et al 2000), the Nordic countries (Hagmar et al 1994), the Czech Republic (Smerhovsky et al 2001), and a black-foot endemic¹ area in Taiwan (Liou et al 1999).

The Italian, Nordic and Taiwan cohorts showed a significant increase in cancer incidence in subjects with a high level of chromosomal aberrations in peripheral blood lymphocytes compared to subjects with a low level of chromosome aberrations. In the Czech cohort a significant association was shown between chromosomal aberrations and cancer in workers exposed to radon, but this association was not seen in workers exposed to various other chemicals. However, the Italian and Nordic cohorts found that neither a high frequency of sister chromatid exchange nor of micronuclei in peripheral blood lymphocytes increased the risk of future cancer (Hagmar et al 1998). Likewise, the black-foot endemic area cohort found no significant difference between the cancer and control groups in the frequencies of sister chromatid exchange in peripheral blood lymphocytes (Liou et al 1999). All four studies suffer from some methodological issues, including possible selection bias, lack of control for confounding and variations in the quality of cytogenetic testing.

Most importantly, the Italian and Nordic cohort studies also found that the increased risk of cancer from a high level of chromosomal aberrations in peripheral blood lymphocytes was independent of exposure to occupational carcinogens and smoking (Bonassi et al 2000). The authors suggested that their results supported a role for individual susceptibility to cancer from genetically determined reduced ability to repair altered DNA.

It is still too early to say that a high level of chromosomal aberrations in peripheral blood lymphocytes (PBL) is associated with an increased risk of future cancer. It is

¹ black-foot disease is associated with arsenic from drinking artesian well water

not likely to be useful as a screening test since it lacks specificity regarding cancer type. Positive findings need to be confirmed. It is not clear whether or not chromosomal aberrations reflect previous exposure to carcinogens or individual susceptibility to cancer from differences in metabolising enzymes or DNA repair capacity. The implications of any association between chromosomal aberrations in PBL and risk of cancer are unresolved.