Immunological analysis of a chromosomal antigen specific to human granulocytes

Karen C. RAMAGE, James H. J. DUNN and Ailsa M. CAMPBELL
Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ, Scotland, U.K.

(Received 28 October 1981/Accepted 31 December 1981)

A chromosomal protein antigen specific for human granulocytes is described. The antigen is present in large amounts in granulocytes and in granulocytic leukaemia. It is absent from HeLa cells, human fibrosarcoma cells and human lymphocytes. It is, however, present in small amounts in other human haemopoietic cell lines. The antigen binds tightly to DNA and requires the presence of DNA to react with the antibody.

Immunological analysis of chromosomal proteins has many applications in both basic and applied research. Antibodies directed against non-histone chromosomal proteins of a specific cell type provide a potential system for the analysis and characterization of those genes that are responsible for determining the phenotype of the cell involved, and several cell-specific chromosomal proteins have been characterized by this method (Campbell *et al.*, 1978, 1979; Briggs *et al.*, 1979a,b; Dunn *et al.*, 1980; McCormack *et al.*, 1982). Specific antiserum to the chromosomes of HeLa cells has been shown to react with no other cell type tested and to have an antigen that is localized in the highly nuclease-resistant area of the chromosomal DNA, sometimes referred to as the scaffold in metaphase cells (Campbell *et al.*, 1979; Dunn *et al.*, 1980). In addition, the binding site has been shown to be present only on human DNA (Dunn *et al.*, 1980). The most successful method for raising such a specific antiserum has been shown to be immunization of the animal with the very small percentage of non-histone chromosomal proteins that remain bound to DNA in the presence of 2M-NaCl/5M-urea at pH6 (dehisonized chromatin). Although this group of proteins may not necessarily always show specificity to cell type, they have been found to do so in rat Novikoff hepatoma (Campbell *et al.*, 1978), HeLa cells (Campbell *et al.*, 1979) and chick erythrocytes (Krajewska *et al.*, 1980).

The potential use of chromosome-specific antibodies in applied research lies in the fact that, although cell-surface antigens may be shared by many cell types, each must possess some chromosomal proteins that confer a unique phenotype. If such proteins can be detected, they could clearly be of diagnostic value. Chromosomal proteins specific to human granulocytes may play a role in the

diagnosis and characterization of some types of leukaemia and in the analysis of the many leukaemic cell lines now available in culture.

In this work the specificity of an antiserum specific to the chromosomal proteins of human granulocytes is examined and the function of the antigen(s) is discussed.

Methods

HeLa cells from the University of Glasgow stock and HT 1080 fibrosarcoma cells were grown in Glasgow modified Eagle's medium supplemented with 10% calf serum. K562 cells (strain S, inducible with either haem or butyrate) were obtained from the Beatson Research Institute, Glasgow, and grown in Ham's F12 medium with glutamate supplemented with 10% foetal-calf serum. Lymphoblastoma cell line GM 1416 was obtained from the Human Genetic Mutant Cell Repository, Camden, NJ, U.S.A., and grown in RPMI medium with glutamine (Gibco) supplemented with foetal-calf serum. Human buffy coats were obtained from the West of Scotland Blood Transfusion Service. Cells from a male patient with chronic granulocytic leukaemia were obtained from Dr. Jon Waters, Department of Genetics, University of Glasgow, and shown by karyotype to have the characteristic Philadelphia translocation. Cells from a male patient with chronic lymphocytic leukaemia were obtained from Dr. G. A. Macdonald, Department of Haematology, Royal Infirmary, Glasgow. All leucocyte samples were purified on Ficoll Paque (Pharmacia).

Chromatin and dehisonized chromatin were prepared as described by Campbell *et al.* (1979). Although the antigen used for immunization of the rabbits was chromatin extracted with 2M-NaCl/5M-urea (Campbell *et al.*, 1978, 1979), whole

chromatin was routinely employed in the specificity assays. Assays were by the micro-complement-fixation method of Wasserman & Levine (1961), although much of the data have been subsequently confirmed by solid-phase binding assay (McCormack *et al.*, 1982). Where low values of anti-complementarity were experienced, they were subtracted from the test samples. Extensive anticomplementarity was not experienced. In several experiments the serial dilution of antigen was on a logarithmic scale so that a wider comparison of antigens could be made.

Chromatin digestion and reconstruction with nuclease and DNA were as described by Dunn *et al.* (1980).

Results

Fig. 1 shows the reaction of granulocyte-specific antiserum with human granulocytes and lymphocytes. The serum shows complete specificity to the granulocyte chromatin. Essentially similar results were obtained with material from a wide variety of human patients, indicating little or no variation in the population. It is clearly of interest to determine whether the specificity is maintained in several cell lines, and in particular with less well differentiated cells in the haemopoietic system. This might indicate whether the chromosomal antigen is produced during granulocyte differentiation or is present in

common stem-cell precursors and simply lost by cells undergoing a different developmental pathway. In Fig. 2 a variety of cell lines is compared at a higher concentration of both antigen and antibody. Sheep granulocytes show no reaction indicating species specificity in the chromosomal antigen. Cells from a patient with granulocytic leukaemia show a strong positive reaction indicating that the antigen may be present not only in terminally differentiated granulocytes but also in myelocytic precursors. Chromatin from the HeLa cell line and from the human fibrosarcoma line HT 1080 shows little reactivity, as does the K562 cell line, which was originally isolated from a patient with chronic myeloid leukaemia in blast crisis but which exhibits strong erythroid characteristics (Anderson *et al.*, 1979). However, the human lymphoblastoma line GM 1416 shows some degree of positive reaction. It seems possible therefore that this line expresses the chromosomal antigen, at least to some extent. The reaction of chromatin from the lymphoblastoma cells was examined over a wider range of DNA concentrations as shown in Fig. 3; this reaction is small compared with that of granulocytes, and several orders of magnitude lower. Fig. 3 also shows the reaction of the chromatin of the lymphocytes of a patient with chronic lymphocytic leukaemia with a lymphocyte count in excess of $200 \times 10^6/\text{mm}^3$. A slight positive response is

Fig. 1. Reaction of granulocyte (●) and lymphocyte (Δ) chromatin with granulocyte specific antiserum

The serum dilution was 1:400. The concentration of chromatin DNA was determined by the A_{260} in 5M-urea, by using a value of $A_{1\text{cm}}^{1\%}$ of 200. Values are means of duplicate determinations in the experiment shown. All experiments were reproducible.

Fig. 2. Reaction of granulocyte specific antiserum with chromatin from other cell types

Chromatin from: ●, human granulocytes; ▲, human chronic granulocytic leukaemia; ○, lymphoblastoma GM1416; □, K562 human granulocytic and erythroid cells; ▽, human HT 1080 fibrosarcoma cells; △, HeLa cells; ■, sheep granulocytes. Growth conditions are given in the Methods section.

Fig. 3. Reaction of granulocyte specific antiserum with chromatin from lymphocytic cells

Chromatin from: ●, granulocytes, ○, chronic lymphocytic leukaemia; △, lymphoblastoma GM 1416. The serum dilution was 1:400.

Fig. 4. Digestion and reconstruction of granulocyte-specific antigen with human DNA

●, Granulocyte chromatin; △, granulocyte chromatin treated with 3000 units of micrococcal nuclease (1 unit gives an increase of 1.0 in A_{260} of acid-soluble material); ○, granulocyte chromatin treated with 3000 units of micrococcal nuclease and reconstituted with the original amount of human placental DNA. Human DNA alone shows no activity in complement fixation. The serum dilution was 1:400.

shown at the highest concentration of antigen. However, more than two orders of magnitude separate the reaction of the chromatin systems.

In Fig. 4 the reaction of chromatin from human granulocytes with large amounts of micrococcal

nuclease is shown. The nuclease abolishes antigenic activity, but this can be completely recovered by the addition of human DNA to the digested material.

Discussion

Among the morphological features that distinguish granulocytes from the other cell types tested are their characteristic lobate nuclei. Functionally they are active phagocytes. They clearly therefore display distinctive phenotypic characteristics, which may be expected to be controlled at the chromosomal level. Immunocytochemical evidence has been presented previously to indicate that nuclear proteins from human lung can show strong tissue-specificity and that this specificity resides largely with the granulocytes of the lung (Briggs *et al.*, 1979b). It may well be expected, however, that human lung, which contains a very large number of cell types, may produce an abnormal antigen through multiple associations. The data presented here show that a nuclear antigen is present in human granulocytes and their tumours in amounts many magnitudes greater than in any other human cells. The reaction of the antiserum described with chromatin from other cell types would appear to confirm the observations made on the cell-surface proteins of the K562 cell line (Anderson *et al.*, 1979). Despite the manifestation of the Philadelphia chromosome translocation, the K562 cell does not possess the characteristic chromosomal antigen present in normal and leukaemic granulocytes. The antigen can, however, be detected to a small degree in lymphoblastic and lymphocytic cells, which suggests either that the potential is present on the common precursor cell and is expressed to some extent in all of its progeny, or that some degree of dedifferentiation has occurred in the lymphocytic cell types tested. Although cell-surface markers that differentiate between some types of leukaemic cells have been described, it is clear that nuclear markers, which have largely been ignored in this field, may have a considerable role to play in the identification of tumour-cell origin.

Any protein that retains strong binding capacity for DNA under the extreme conditions of salt extraction employed for preparation of the primary antigen is of obvious interest in the field of DNA-protein interactions. The fact that the protein can be dissociated from DNA by very high concentrations of micrococcal nuclease and subsequently reconstituted with DNA with a recovery of antigenic activity confirms that a true DNA-binding protein is involved. Strong binding is a characteristic expected for proteins that control gene expression and is shown by bacterial control proteins. However, if an analogy with bacterial

systems is to be made, very small amounts of such proteins would be expected to be present in chromatin. The comparatively great extent of the reaction of the granulocyte material would suggest that considerable amounts of the antigen are present. Consequently it may be possible that the antigen is responsible for the activation of a large number of genes, that it is made in excess and has a broad DNA-binding specificity, or that it has some structural role manifested in granulocyte chromatin alone. None of these possibilities reflects a role for chromosomal proteins as yet described in the literature of chromosomal proteins, and there is therefore much potential for further biochemical analysis.

This work was supported by the Science and Engineering Research Council, by the Medical Research Council and by the Cancer Research Campaign.

References

- Anderson, L. C., Nilsson, K. & Gahmberg, C. G. (1979) *Int. J. Cancer* **23**, 143–147
- Briggs, R. C., Campbell, A. M., Chiu, J. F., Hnilica, L. S., Lincoln, G., Stein, J. & Stein, G. (1979a) *Cancer Res.* **39**, 3683–3688
- Briggs, R. C., Chiu, J. F., Hnilica, L. S., Rogers, L. W. & Page, D. L. (1979b) *Cell Differ.* **7**, 313–323
- Campbell, A. M., Briggs, R. C., Zimmer, M. S., Krajewska, W. M., Chiu, J. F. & Hnilica, L. S. (1978) in *Biological Markers of Neoplasia* (Ruddon, R. W., ed.), pp. 369–384, Elsevier, Amsterdam
- Campbell, A. M., Briggs, R. C., Bird, R. E. & Hnilica, L. S. (1979) *Nucleic Acid Res.* **6**, 205–218
- Dunn, J. H. J., Lyall, R. M., Briggs, R. C., Campbell, A. M. & Hnilica, L. S. (1980) *Biochem. J.* **185**, 277–279
- Krajewska, W. M., Briggs, R. C., Chiu, J. F. & Hnilica, L. S. (1980) *Biochemistry* **19**, 4667–4673
- McCormack, M., Dunn, J. H. J. & Campbell, A. M. (1982) *J. Immunol. Methods* in the press
- Wasserman, E. & Levine, L. (1961) *J. Immunol.* **87**, 290–295