21. Pal, A., Stokes, D. L., Alarie, J. P. and Vo-Dinh, T., Selective surface enhanced Raman spectroscopy using a polymer-coated substrate. *Anal. Chem.*, 1995, **67**, 3154–3159.
22. Tsang, J. C. and Kirtley, J., Anomalous surface enhanced molecular Raman scattering from inelastic tunneling spectroscopy junctions. *Solid State Commun.*, 1979, **30**, 617–620.
23. Kobayashi, Y. and Itoh, K., Surface-enhanced resonance Raman scattering spectra of meso-substituted porphines in layered structures having calcium fluoride/porphine/silver and calcium fluoride/silver/porphine configurations. *J. Phys. Chem.*, 1985, **89**, 5174–5178.
24. Vo-Dinh, T., Hiromoto, M. Y. K., Begun, G. M. and Moody, R. L., Surface-enhanced Raman spectrometry for trace organic analysis. *Anal. Chem.*, 1984, **56**, 1667–1670.
25. Szabo, N. J. and Winefordner, J. D., Evaluation of a solid-phase extraction membrane as a surface-enhanced Raman substrate. *Appl. Spectrosc.*, 1998, **529**, 500–512.
26. Akbarian, F., Dunn, B. S. and Zink, J. I., Surface-enhanced Raman spectroscopy using photodeposited gold particles in porous sol-gel silicates. *J. Phys. Chem.*, 1995, **99**, 3892–3894.
27. Yonezawa, Y., Sato, T. and Kawabata, I., Photo-induced formation of gold metal film from metal salt of chitosan. *Chem. Lett.*, 1994, 355–358.
28. Chumanov, G., Sokolov, K., Gregory, B. W. and Cotton, T. M., Colloidal metal films as a substrate for surface-enhanced spectroscopy. *J. Phys. Chem.*, 1995, **99**, 9466–9471.
29. Weaver, S., Taylor, D., Gale, W. and Mills, G., Photoinitiated reversible formation of small gold crystallites in polymer gels. *Langmuir*, 1996, **12**, 4618–4620.
30. Gross, M. E., Fisanick, G. J., Gallagher, P. K., Schnoes, K. J. and Fennel, M. D., Laser-initiated deposition reactions: microchemistry in organogold polymer films. *Appl. Phys. Lett.*, 1985, **47**, 923–925.
31. Ye, Y. and Hunsperger, R. G., Ultraviolet-light-induced deposition of gold films. *Appl. Phys. Lett.*, 1987, **51**, 2136–2138.
32. Yonezawa, Y., Takami, A., Sato, T., Yamamoto, K., Sasanuma, T., Ishida, H. and Ishitani, A. J., Photochemical formation of silver metal films from silver salt of natural high molecular carboxylic acid. *J. Appl. Phys.*, 1990, **68**, 1297–1302.
33. Yonezawa, Y., Kijima, M. and Sato, T., Aggregation of silver particles in silver alginate films. *Ber. Bunsenges. Phys. Chem.*, 1992, **96**, 1828–1831.
34. Yonezawa, Y., Miyama, T. and Sato, T., Transfer of dye monolayers onto silver metal films formed by photolysis of silver salt of carboxymethylcellulose. *Chem. Lett.*, 1992, 2455–2458.
35. Bell, S. E. J., McGarvey, J. J., Rigby, S. J. and Walmsley, D. G., Raman scattering and scanning electron tunneling studies of metal liquid-like films produced from silver and gold sols. *J. Raman Spectrosc.*, 1991, **22**, 763–769.
36. Giersig, M. and Mulvaney, P., Formation of ordered two-dimensional gold colloid lattices by electrophoretic deposition. *J. Phys. Chem.*, 1993, **97**, 6334–6336.
37. Halpern, B. L., Fast flow deposition of metal atoms on liquid surfaces: gold sol formation and surface lifetime. *J. Colloid Interface Sci.*, 1982, **86**, 337–343.
38. Esumi, K., Suzuki, A., Aihara, N., Usui, K. and Torigoe, K., Preparation of gold colloids with UV irradiation using dendrimers as stabilizer. *Langmuir*, 1998, **14**, 3157–3159.
39. Balogh, L. and Tomalia, D. A., Poly(amidoamine) dendrimer-templated nanocoposites. 1. Synthesis of zerovalent copper nanoclusters. *J. Am. Chem. Soc.*, 1998, **120**, 7355–7356.
40. Zhao, M., Sun, L. and Crooks, R. M., Peparation of Cu nanoclusters within dendrimer templates. *J. Am. Chem. Soc.*, 1998, **120**, 4877–4878.
41. Pal, A., Photochemical formation of gold nanoparticles in aqueous Triton X-100 and its application in SERS spectroscopy. *Curr. Sci.*, 1998, **74**, 14–16.
42. Pal, A., Photoinitiated gold sol generation in aqueous Triton X-100 and its analytical application for spectrophotometric determination of gold. *Talanta*, 1998, **46**, 583–587.
43. Pal, A., Photochemical dissolution of gold nanoparticles by bromine containing trihalomethane (THMs) such as bromoform in aqueous Triton X-100 medium and its analytical application. *J. Photochem. Photobiol. A: Chem.*, 2001, **142**, 59–65.
44. Sau, T. K., Pal, A., Jana, N. R., Wang, Z. L. and Pal, T., Size controlled synthesis of gold nanoparticles using photochemically prepared seed particles. *J. Nanopart. Res.*, 2001, **3**, 257–261.
45. Mandal, M., Kundu, S., Ghosh, S. K., Jana, N. R., Panigrahi, M. and Pal, T., Sniffing a single molecule through SERS using Au core-Ag shell bimetallic nanoparticles. *Curr. Sci.*, 2004, **86**, 556–559.
46. Isola, N. R., Stokes, D. L. and Vo-Dinh, T., Surface-enhanced Raman gene probe for HIV detection. *Anal. Chem.*, 1998, **70**, 1352–1356.
47. Vo-Dinh, T., Houck, K. and Stokes, D. L., Surface-enhanced Raman gene probes. *Anal. Chem.*, 1994, **66**, 3379–3383.
48. Narayanan, V. A., Stokes, D. L. and Vo-Dinh, T., Vibrational spectra of the industrial dyes cresyl fast violet, phloxine B and safffron. Intensity studies by surface-enhanced Raman spectroscopy. *Analysis*, 1996, **24**, 1–5.
49. Pal, T., Narayanan, V. A., Stokes, D. L. and Vo-Dinh, T., Surface-enhanced Raman detection of nicotinamide in vitamin tablets. *Anal. Chim. Acta*, 1998, **368**, 21.

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In vitro lymphocyte-to-granulocyte transdifferentiation induced by chemicals

B. V. Shenoi

Kamath Laboratory, Geetha Road, Robertsonpet,
Kolar Gold Field 563 122, India

Transdifferentiation refers to a physiologic process of alteration of one distinct phenotypic cell into another during development and differentiation. The concept that the small lymphocyte is capable of undergoing transdifferentiation into other cell types, has long been envisaged. The present communication presents cogent evidence of lymphocyte transdifferentiation into granulocytes induced *in vitro* by sodium nitroprusside, a nitric oxide donor. This conclusion is substantiated by the cytochemical demonstration of myeloperoxidase, an acknowledged biological marker in transforming lymphocytes. This finding engenders a significant concept unifying circulating leucocytes besides intergrating lymphocytes into the mainstreams of both haematology and immunology.

EVEN after a lapse of well over two hundred years since their discovery by William Hewson in the 1770s, lympho-

e-mail: bvshenoi@yahoo.com

cytes still remain enigmatic cells wrapped in mystery. Consequently, current knowledge concerning their origin, function and fate remains imprecise.

A challenging question confronting haematologists since the time Paul Ehrlich (the founder of modern haematology) introduced differential staining of blood smears in 1879, has been whether all leucocytes can be placed 'under one roof' or not? As yet, no convincing evidence has been forthcoming to provide an answer to this crucial question with absolute finality.

After pioneering investigation, Yoffey and Courtice[1] arrived at the far-sighted conclusion that the small lymphocyte is a specialized circulating form of the primitive mesenchymal cell capable of development into other cell types in response to stimuli as yet unknown. This concept was lying dormant until the quest was taken up by us in 1968.

The main objective of the heuristic study was to identify plant lectins, if any, capable of inducing lymphocyte transdifferentiation *in vitro*. Soon two potential plant lectins providing us with a glimpse of lymphocyte transdifferentiation into granulocytes were identified[2–4]. However, further progress was thwarted by flaws in the methodology besides absence of characterization and standardization of lectins employed.

Lately, by a fortuitous coincidence, sodium nitroprusside (SNP), a nitric oxide donor was discovered to induce consistently successful granulocytic transdifferentiation *in vitro*. *In vitro* lymphocyte activation employing SNP for inducing cell signalling was serendipitous, based on the reported observations of Park *et al.*[5].

Our study group comprised one hundred apparently healthy individuals aged 25–50 years (75 males and 25 females). Fasting blood samples obtained by venepuncture were collected in EDTA anti-coagulant. Lymphocyte separation was carried out without any delay, in aliquots of 2.0 ml by gradient density centrifugation procedure described by Boyum[6], employing Histopaque (R)-1077 (Sigma). After centrifugation, the opaque layer comprising mainly lymphocytes, other mononuclears and platelets, measuring invariably about 1.0 ml was carefully aspirated and delivered into a centrifuge tube. The step involving phosphate-buffered saline solution wash was entirely eliminated, essentially for the preservation of platelets for subsequent interaction. Next 40.0 µl of a 1.0 mM solution of SNP was then added and allowed to stand at room temperature for 30 min, after which the tube was centrifuged and thin smears were prepared from the sediment. A wet mount was also made for simultaneous study of living cytology. Air-dried smears were stained with haematoxylin-eosin and Leishman's stain. Peroxidase-staining was performed by the following method: (i) Benzidine reagent – 0.3 g of benzidine dissolved in 95% absolute ethyl alcohol to which a few drops of saturated SNP are added. This reagent is stable for months. (ii) Dilute hydrogen peroxide is prepared by adding 10 drops of 40 volumes per cent hydrogen peroxide to 25.0 ml of distilled water to be prepared fresh daily. Air-dried smears are covered with 1.5 ml of benzidine reagent and allowed to stand for 2 to 3 min after which 0.8 ml of diluted hydrogen peroxide solution is added directly and allowed to interact for 3 to 4 min, washed with distilled water and counterstained with 2% aqueous eosin or saturated picric acid.

The results of the chemical cell-signalling study were impressive. The technique was direct, reliable and reproducible. Earlier, a search of *Medline* for articles on lymphocyte transdifferentiation had proved futile.

For want of appropriate facilities to identify cell-surface markers, the main criterion on which our evidence rests is cytomorphology together with peroxidase positivity. Both of these, therefore, respectively, identify the weakness and the strength of our study. Cell-surface markers would have indeed facilitated the identification of the responding subpopulations of lymphocytes.

Cell signalling sets in motion a series of cytomorphological events for which the presence of platelets appears to be a basic pre-requisite. Our own earlier study had led us to this conclusion[4]. The precise role of platelets, however, remains speculative. Platelet-derived growth factor in concert with cytokines presumably plays a key role in these events, culminating in transdifferentiation.

Significant events occurring synchronously are platelet adhesion, lymphocyte activation, migration and adhesion. Stained smears consistently exhibit an overall increase in cell volume, nuclear eccentricity, appearance of 'hand-mirror' forms, nuclear indentation followed by segmentation.

Peroxidase-stained smears remarkably display the entire gamut of enzyme activity ranging from its earliest, incipient stages to complete, dense granulation filling the entire cell. The different counterstains employed by us enhanced and highlighted the efficacy of the method. Figure 1 *b–f* clearly depicts the initial appearance of an invariably central dark staining dot heralding the appearance of the enzyme marker followed thereafter by a reticulated appearance progressing rapidly to dense intracellular granulation.

Both on the observations derived from a hundred blood samples included in the present study besides others which were incidentally studied, the percentage of myeloperoxidase (MPO)-positive cells in any given sample after stimulation with SNP could not be estimated precisely owing mainly to cell aggregation after stimulation, leading to an uneven spread of cells in stained smears of the sedimented cells. Further, harvested lymphocytes prior to stimulation invariably contain 2–3% granulocytes despite great care taken to minimize such contamination. Inevitably, therefore, these two unavoidable factors would be expected to result in some degree of error in quantifying transdifferentiation based on a smear study. Nevertheless,

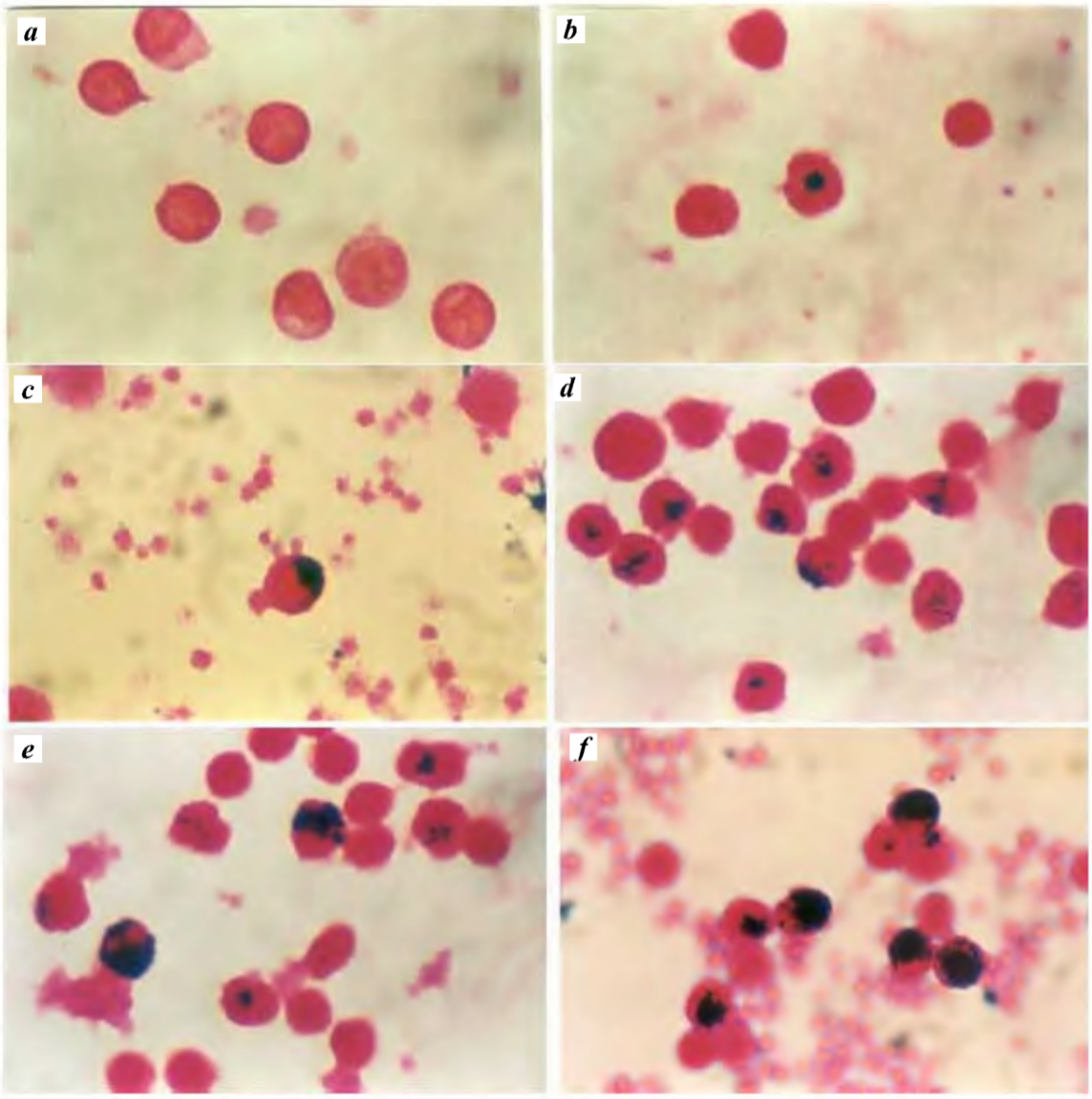

Figure 1. ***a***, Control (untreated): Small and medium lymphocytes with a few scattered platelets in the background. 2% aqueous eosin counterstain (×1000). ***b–f***, Treated smears. ***b***, Lymphocyte in the centre displays a clearly-defined central large black dot representing early peroxidase enzyme activity. ***c***, Lymphocyte shows 'hand-mirror' form the adherent platelets and small platelet-aggregates are seen in the background. ***d***, Lymphocyte adhesion with intercellular bridging exhibiting various degrees of peroxidase positivity. ***e***, Platelet aggregates with lymphocyte adhesion and intercellular bridging, peroxidase activity ranging from early to complete. Peroxidase stain with eosin counterstain (×1000) for ***b–d***. ***f***, Lymphocyte and platelet aggregates; most lymphocytes exhibit marked peroxidase positivity (×1000).

judging on the basis of overall density of MPO-positive cells in the smears studied, on an average, approximately 30 to 50% of the stimulated cells *in vitro* exhibit a positive response signifying transdifferentiation.

In conclusion, granulocytic transdifferentiation of lymphocytes therefore integrates not only all the circulating leucocytes, but also haematology and immunology with consequent far-reaching changes in perceptions and perspectives in our understanding of a whole gamut of disease processes.

1. Yoffey, J. M. and Courtice, P. C., In *Lymphatics, Lymph and Lymphoid Tissue*, Edward Arnold Ltd, London, 1956, 2nd edn, p. 392.
2. Shenoi, B. V., The action of two different lectins on avian and mammalian cells – some interesting observations. Paper presented at the XXXV Annual Conference of the Association of Pathologists and Microbiologists of India, Bangalore, 27–30 December 1986.
3. Shenoi, B. V., Lymphocyte transformation – a myth or a reality? *Spec. Sci. Technol.*, 1988, **II**, 215–217.
4. Shenoi, B. V., Granulocytic differentiation of human peripheral blood lymphocytes induced by a platelet-euphorbia lectin complex

in vitro: a preliminary communication. The Immunologist, Abstr. 10th International Conference of Immunology, New Delhi, 1–6 November 1998, p. 624.
5. Park, Raekil, Young-Choi, D. U., Kim, Myung-Sun, Soh, Hong-sub, Jung, Byung-Hak, Jun, ChangDuk and Chung, Hun-Taeg, Bacterial endotoxin, lipopolysaccharide, induced the activation of stress – activated protein kinase in RAW 264.7 cells. The Immunologist, Abstr. 10th International Conference of Immunology, New Delhi, 1–6 November 1998, p. 286.
6. Boyum, A., Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest.* (*Suppl.*), 1968, **21**, 77.

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CAGCAG – the most consistent repeating pattern in evolution of small subunit of rRNA gene sequences

D. S. Iyer, D. V. Raje, H. J. Purohit*, A. Gupta and R. N. Singh

National Environmental Engineering Research Institute, Nehru Marg, Nagpur 440 020, India

Conserved patterns in nucleotide sequences are often suspected for their possible structural or functional implications. In this exercise, repeating patterns of nucleotides of size six or more that are conserved in rRNA sequences of three evolutionary domains, have been targetted. The pattern CAGCAG was found to be the most consistent repeating pattern in 16S rRNA of *Proteobacteria* and 18S rRNA of *Eucarya*, but the repetitiveness was not observed in *Archaea*. This pattern or the residues within have not been reported for their biological relevance; but still the information contained between the repeats of the pattern was found to be of much relevance in classification using similarity and multiple discriminant analysis.

THE universal phylogenetic tree of life has been proposed based on small subunit of rRNA gene sequences using alignment techniques. Three lines of evolutionary descent, viz. *Eucarya* (eukaryotes), *Proteobacteria* and *Archaea* have been explored thoroughly[1,2]. Further, it has been shown that the residues of small subunit rRNA molecule play a crucial role in protein synthesis[3]. Amongst different small subunit rRNAs, 16S rRNA has been extensively explored for its association with bacterial diversity[4] and also for its functional role in protein biosynthesis[5]. The 16S rRNA is found in the 30S sub-unit of the ribosome, which has similar secondary structure with its counterpart 18S rRNA in the 40S subunit of the ribosome in eukaryotes[6].

Analysing the genetic information in terms of patterns or identifying regions that are preserved during evolution and relating the findings with structure and function of a gene, are issues of immense interest since the last decade. There are some deterministic pattern-discovery algorithms available, which can find sparse amino or nucleic acid patterns matching in protein or DNA sequences[7–9]. The origin, evolution and distribution of repetitive elements in genome sequences have been studied, both experimentally and computationally. There are programs available for identifying such repeated patterns in large genome sequences and identify repeating patterns of size at least 20 bases as mini or micro satellite information to characterize DNA. Amongst these, the recently developed REPuter (http://bibiserv.techfak.uni-bielefeld.de/reputer) has been found to be efficient and provides exhaustive repeats in sequences[9]. Although the programs provide the list of repeats in a sequence, they do not have an option to automatically provide repeating patterns, which are conserved across the input set of homologous sequences by considering the separating distance criterion. We have developed a program, Repeat Tuple Search (RTSearch; www.ebi.ac.uk/~lijnzaad/RepeatTupleSearch), which has this additional feature to determine the consistent repeating patterns (CRPs) across the set of sequences. By consistent, we mean the repeating pattern (length at least six bases) occurring across majority of the input sequences, such that the separating distance between the two repeats in these sequences remains constant. The repeats are exact and do not allow even single base ambiguity. The program works efficiently for small sequences of up to size 2 kb. It has two basic components – the first determines repeating patterns of length more than six (default setting) along with the separating distance between the repeats in each input sequence. The search for repeating patterns is exhaustive, without asking for any input conditions from the user. The second component processes the collective data to get the number of sequences in which different repeating patterns make their appearances, considering the constant separating distance criterion. Patterns with high frequency of occurrence are considered as CRPs.

Earlier, we had reported that four repeating patterns occur with more than 80% consistency across the sampled set of fifty different 16S rRNA sequences of *Pseudomonas*, with CAGCAG being the most consistent repeating pattern. The sub-sequences between the repeats were analysed using information theory to obtain the signature for genus

*For correspondence. (e-mail: hemantdrd@hotmail.com)