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HIGH-RESOLUTION TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS OF ACIDIC, BASIC AND SURFACE PROTEINS OF MOUSE NEUTROPHILS

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Summary

The proteins from murine neutrophils have been examined using isoelectric focusing and non-equilibrium pH gradient electrophoresis in the first dimension and sodium dodecyl sulfate-polyacrylamide electrophoresis as a second dimension. The major protein, actin, dominates the protein profiles and it appears to be one of the few proteins being synthesised rapidly. In the presence of protease inhibitors, neutrophil (a homogeneous, non-dividing cell population) lysates gave extremely reproducible two-dimensional electrophoretic patterns both with Coomassie blue staining (approx. 200 proteins detected) and with fluorography or autoradiography after [^{35}S]methionine biosynthetic labelling (approx. 450 proteins detected between pH 4 and 7). Biosynthetic labelling was more sensitive than protein staining for some components, although the mature neutrophils did not synthesis certain cellular proteins (e.g., granule proteins such as lactoferrin). Surface labelling of neutrophils (as indicated by the absence of ^{125}I associated with actin) yielded more than 20 major ^{125}I-labelled proteins on high-resolution electrophoretic maps. The major ^{125}I-labelled protein (M_r ~ 90 kdalton) focused at the acidic end of the gels near pH 4.1. This protein could also be detected after [^{35}S]methionine biosynthetic labelling. All of the high molecular weight components focused over a broad pH range (0.2 pH units). At lease one of the surface components appeared to consist of several discrete charge entities.

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Abbreviations; DMSO, dimethyl sulfoxide; PPO, 2,5-diphenyloxazole.

Introduction

Hemopoietic differentiation has been studied at a cellular level with considerable success but, until recently, approaches to the molcular events have been mainly restricted to erythropoiesis [1,2]. Cells of the myeloid series are known to develop from a set of progenitors [3] under the stimulation of granulocyte-macrophage colony stimulating factor [4] and it is now possible to obtain highly purified preparations of murine polymorphonuclear neutrophils [5] so that the total and surface proteins of the end stage of differentiation can be analysed in detail. Indeed, neutrophils provide one of the few non-dividing homogeneous cell populations suitable for characterisation by high-resolution two-dimensional gel electrophoresis [6,7]. The protein patterns should be quite reproducible: whether static, as determined by Coomassie blue staining, or dynamic, as determined by [^{35}S]methionine incorporation. Similar patterns from tumor cell lines or heterogeneous cell populations, e.g., lymphocytes, may well be difficult to quantitate and reproduce meaningfully. There are several tumor cell lines which appear to be responsive to the colony stimulating factor [8,9], but initial attempts to analyse protein changes induced during differentiation have been rendered difficult [10] by a lack of knowledge of the protein profile for any normal cells in the myeloid series. In particular, it is not a simple task to distinguish between the proteins arising naturally during myeloid differentiation and those associated with the proliferation of the tumor cells.

We have undertaken the high-resolution two-dimensional electrophoretic analysis of the murine neutrophil proteins using isoelectric focusing, non-equilibrium pH gradient electrophoresis and SDS-polyacrylamide gel electrophoresis [6,7]. Neutrophils are mature, functional leukocytes in which the rate of protein [5] and nucleic acid synthesis [11] appears to be slower than that in many other cell types. Many of the granular proteins (e.g., lactoferrin) are thought to be synthesised at early stages of myeloid development [12] so our electrophoretic analyses have been developed using both direct protein staining [13] and [^{35}S]methionine biosynthetic labeling and autoradiography. The surface proteins of neutrophils were labeled [5] using 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril [14] and analysed using two-dimensional electrophoresis. The results are compared to the surface labeling characteristics of other mammalian cell types.

Materials and Methods

Chemicals, buffers and solutions

^{125}I (100 Ci/mmol) and [^{35}S]methionine (800 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, Bucks., U.K. Iodogen ® (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril) was obtained from Pierce, Rockford, IL, U.S.A. SDS, *N,N,N*^{1},*N*^{1}-tetramethylethylenediamine (Temed), EDTA (disodium salt) and $(NH_4)_2S_2O_8$ were bought from British Drug House Chemicals Ltd., Poole, U.K. Percoll and the molecular weight markers for electrophoresis were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. The molecular weight marker proteins for gel electrophoresis were prepared

as stock solutions of 0.5 mg/ml in SDS sample buffer : glycerol (7.5%), SDS (1.5%), 2-mercaptoethanol (2.5%) in 0.034 M Tris-HCl (pH 6.8). Acrylamide, N,N^1-methylenebisacrylamide, Kodak RP/S X-Omat and Industrex-A films were purchased from Eastman-Kodak, Rochester, NY, U.S.A. Calcium caseinate (casein) was obtained from Glaxo Laboratories, Australia. Phenylmethylsulfphonyl fluoride was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Dulbecco's modified Eagle's medium was bought from Grand Island Biological Co., NY, U.S.A., and methionine-free Dulbecco's modified Eagle's medium from the Commonwealth Serum Laboratories, Parkville, Australia. Fetal calf serum was obtained from Flow Laboratories, Australia.

Isolation and radioactive labeling of murine neutrophils

Male C57BL/6f/J WEHI mice (8—12-weeks old), which had been bred under specific pathogen-free conditions and conventionalised at 5 weeks of age, were used. Neutrophils (greater than 97% purity) were obtained from casein-induced peritoneal exudates by density centrifugation on Percoll as described previously [5]. Surface iodination of the cells was mediated by iodogen [5,14].

Purified neutrophils ($2—5 \cdot 10^7$ cells per ml) were biosynthetically labeled in methionine-free Dulbecco's modified Eagle's medium (1 ml) containing dialysed fetal calf serum (5%, v/v) and supplemented with carrier l-methionine (10 μM) and [^{35}S]methionine (250 μCi) for 3 h at 37°C in a humidified atmosphere of 10% (v/v) CO_2 in air [5]. The labeled cells were diluted to 10 ml with serum-free Dulbecco's modified Eagle's medium, chilled for 30 min on ice and washed four times with ice-cold phosphate-buffered saline.

Electrophoretic analysis of neutrophil proteins

Preparation of the cell-free extract. Samples were prepared for isoelectric focusing and non-equilibrium pH gradient electrophoresis using a procedure similar to that described by O'Farrell [6]. Cells were suspended at $2 \cdot 10^8$ cells per ml in Tris-HCl buffer (0.01 M, pH 7.4), freeze-thawed three times and digested with DNAase I (1 μg/ml) in the presence of $MgSO_4$ (0.2 mM). Phenylmethylsulfonyl fluoride (in ethanol) and EDTA were added to the cell-free extract at final concentrations of 20 and 10 mM, respectively, in order to inhibit endogenous proteases. Solid urea was then added to a final concentration of 9.2 M and an equal volume of SDS lysis buffer (9.2 M urea, SDS (1%, w/v), 2-mercaptoethanol (1%, v/v): LKB Ampholines, pH 3.5—10 (0.4%, w/v)) added. After incubation of the cell-free extract at 4°C for 10 min, the isoelectric focusing lysis buffer (9.2 M urea, Triton X-100 (2%, w/v); LKB Ampholines, pH 5—8 (1.6%, w/v) and pH 3.5—10 (0.4%, w/v); 2-mercaptoethanol (0.5%, v/v)) was added (1 vol. cell extract: 1 vol. isoelectric focusing lysis buffer) to dissociate the SDS-protein complexes. The neutrophil protein extracts were stored at —20°C prior to use.

Isoelectric focusing. The isoelectric focusing gel was prepared essentially as described by O'Farrell and O'Farrel [15].

Non-equilibrium pH gradient electrophoresis. Non-equilibrium pH gradient electrophoresis gels were prepared as described by O'Farrel et al. [7].

Measurement of pH gradient. The isoelectric focusing and non-equilibrium pH gradient electrophoresis gels were sliced horizontally and each section

equilibrated for 5 min with 1 ml of degassed deionized water. The pH of the solution was measured using an Ingold surface microelectrode (Type 10-403-3006) attached to an Orion pH meter model No. 701-1A.

Second dimension SDS-polyacrylamide gel electrophoresis. The apparatus used was similar to that described earlier [15]. The slab gels (1 X 140 X 140 mm) were poured at a uniform acrylamide concentration of 12% (w/v) as previously described [5,15,16]. The isoelectric focusing or non-equilibrium pH gradient electrophoresis gel was equilibrated for 30 min, placed on to the second dimension gel and contact secured between the first and second dimension gels by the addition of 1 ml of agarose solution (1%, w/v, in equilibration buffer). The electrophoresis was performed at 20 mA for 4—5 h until the bromophenol blue marker reached the end of the gels. Slab gels were stained with Coomassie brilliant blue R-250 in methanol (50%, v/v) and acetic acid (10%, v/v) [13]. Standard protein markers were obtained from Pharmacia, Uppsala, Sweden.

Autoradiography of electropherograms. Slab gels of cell extracts labeled with [^{35}S]methionine were dried under vacuum onto Whatman No. 1 filter paper and exposed to No-Screen Kodak RP/S X-Omat or Industrex-A film. The gels were autoradiographed at —70°C prior to being developed for 5 min at 20°C in Agfa-Gevaert G-150 X-ray developer and fixed for 3 min at 20°C in Agfa-Gevaert G 334 rapid X-ray fixer.

Fluorography of electropherograms. In order to decrease the time of exposure of the gels to the X-ray film, the gels were prepared for fluorography using the DMSO-PPO method of Bonner and Laskey [17] or by immersing the gels in a 3-fold volume of Enhance (New England Nuclear) for 1 h prior to water precipitation and gel drying.

Results

Two-dimensional separation of neutrophil proteins

The components of mouse peritoneal exudate neutrophils were analysed by two-dimensional gel electrophoresis. The conditions for isoelectric focusing (6800 V · h) and non-equilibrium pH gradient electrophoresis (1600 V · h) produced linear pH gradients. The pH as measured after elution with distilled deionized water ranged from pH 4.1 to 6.7 for the isoelectric focusing gel and from pH 4.1 to 9.5 for the non-equilibrium pH gradient gel.

The isoelectric focusing-SDS protein profile for peritoneal exudate neutrophils is displayed in Fig. 1a. Provided that care was taken with sample preparation, this profile was highly reproducible. In particular, the inclusion of protease inhibitors prevented significant changes to the protein patterns developed in two dimensions. Furthermore, reduction of the viscosity of the sample with DNAase I was essential for maintaining reproducibility of the electrophoretic patterns. However, some variations occurred between the isoelectric focusing and SDS runs. In the complete profile shown in Fig. 1a, 102 individual Coomassie blue-stained polypeptides were easily identified. The murine neutrophil sample analysed contained proteins from $1 \cdot 10^6$ cells. Many more Coomassie blue spots could be detected by applying a lysate containing the equivalent of $5 \cdot 10^6$ cells to the isoelectric focusing gel. Indeed,

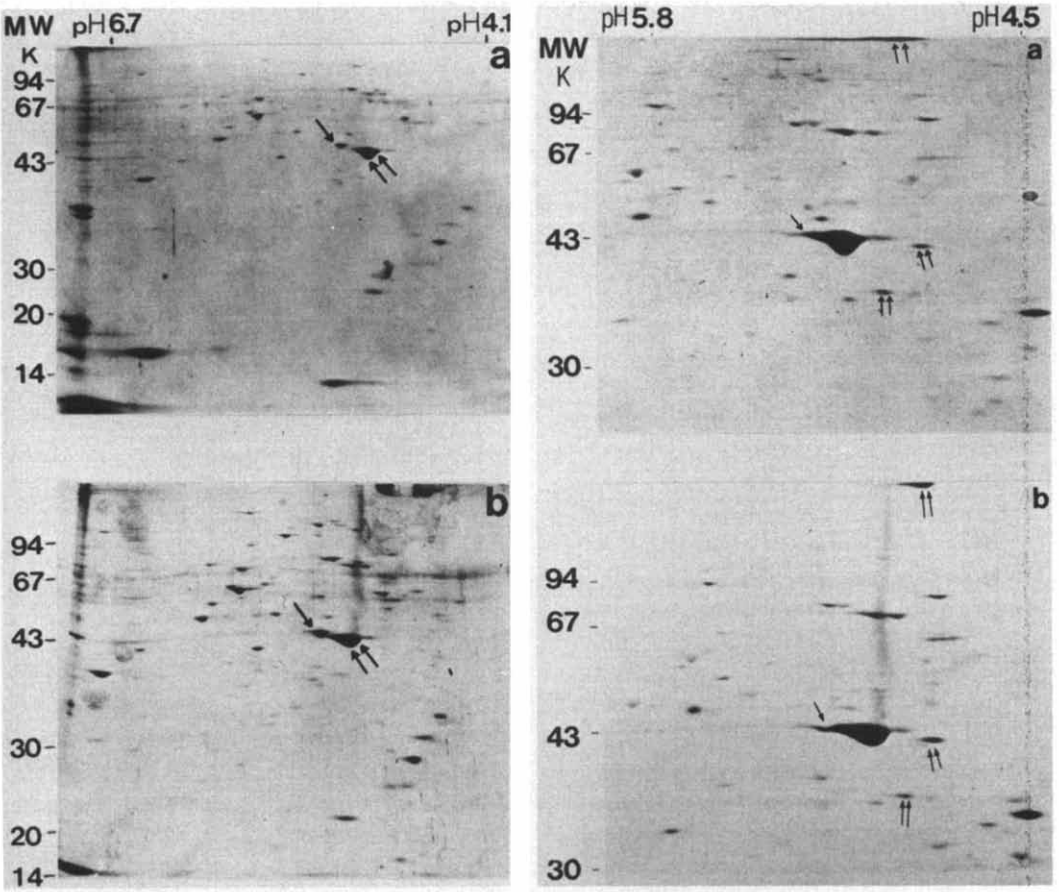

Fig. 1. Separation of [^{35}S]methionine-labeled neutrophil proteins on two-dimensional isoelectric focusing-SDS gels. The effect of increasing the amount of protein applied to the gels on the number of Coomassie blue-stained spots is evident from a comparison of the two maps shown: (a) $5 \cdot 10^5$ cells per gel; (b) 10^6 cells per gel. Some protein spots are artifactual in b. The slab gel was 12% acrylamide. The protein, actin, is indicated by double arrows. The major component indicated by the single arrow is not labeled biosynthetically under the conditions used.

Fig. 2. Separation of [^{35}S]methionine-labeled neutrophil proteins using isoelectric focusing-SDS gel electrophoresis (as for Fig. 1). The dried gels were exposed to RP/S X-OMAT film for 28 days at —70°C. A section of each autoradiograph is shown. Several proteins which appear on the Coomassie blue-stained gels (Fig. 1) are absent from the autoradiographs. The absence of one major component is indicated by the single arrow. Several ^{35}S-labeled components (double arrows) do not appear on the Coomassie blue profiles. (a) $5 \cdot 10^5$ cells loaded per gel, (b) 10^6 cells loaded per gel.

220 Coomassie blue spots were now evident (Fig. 1b). Some spots, however, appeared to be artifactual, presumably because of incomplete equilibrium between the SDS and particular focused proteins. This was evident when the ^{35}S autoradiograms of the gels presented in Fig. 1 were examined (Fig. 2a and b). A group of proteins apparently with identical isoelectric points (slightly more acidic than actin) formed several bands of different apparent molecular weights in the SDS-polyacrylamide gel. Such a spotting pattern was almost certainly artifactual, since these proteins were not present in five replicate

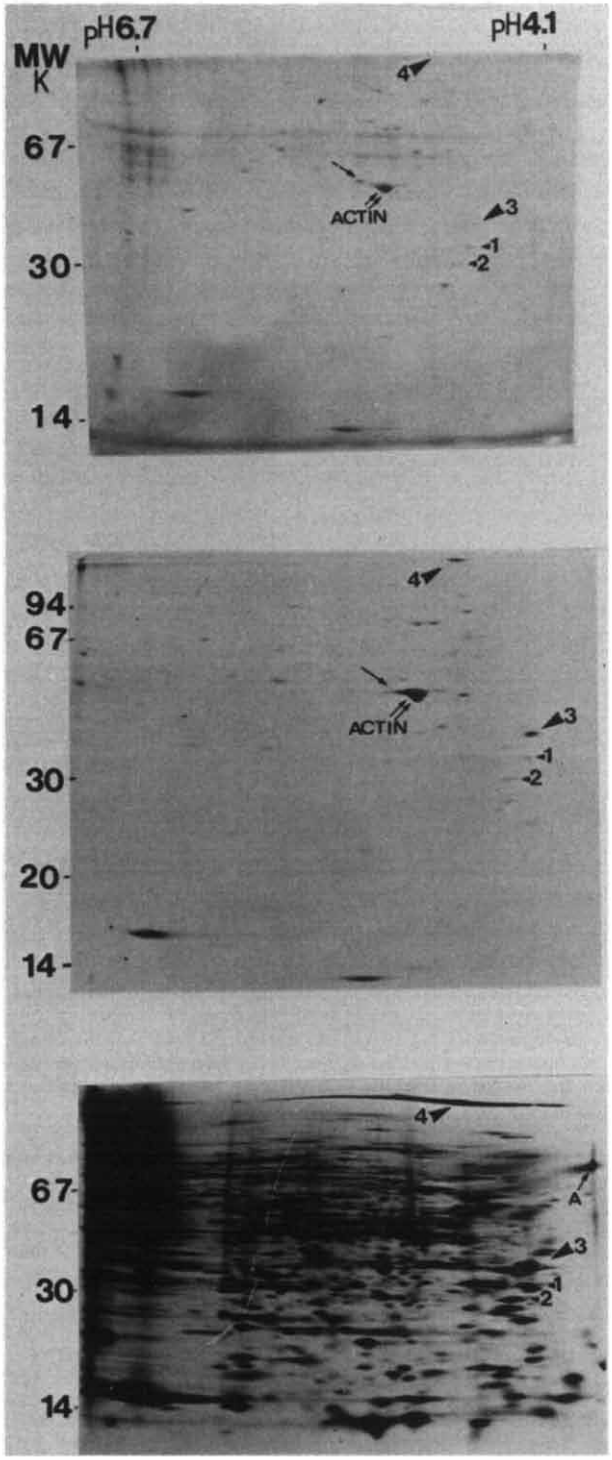

Fig. 3. Separation of [^{35}S]methionine-labeled neutrophil proteins using two-dimensional isoelectric focusing-SDS gels. (a) Coomassie blue-stained proteins; (b) autoradiograph was exposed to RP/S X-OMAT film for 10 days at —70° C; (c) autoradiograph exposed for 42 days. Single arrow indicates one Coomassie blue-stained component which did not label with [^{35}S]methionine. Proteins labeled 1 and 2 stained with Coomassie blue and were detectable with autoradiography whereas proteins 3 and 4 were only detectable by autoradiography.

experiments with lower protein loadings. Overloading of a particular protein generally did not lead to artifactual bands (e.g., actin), but often a diffuse streak of the Coomassie blue-stained and ^{35}S-labeled material was present from the top of the gel to the position of the overloaded component (Fig. 2b). Minor polypeptide bands appeared to focus independently of these artifacts so that overloading in one isoelectric focusing region (e.g., Fig. 2b) did not affect the focusing in other regions (Fig. 1a and b). Even proteins which focused identically with actin appeared to migrate as homogeneous spots in the SDS-polyacrylamide gel (Fig. 2a and b). Charge microheterogeneities, resulting from sample preparation, were not observed.

Actin (M_r 42 000; pI approx. 5.2), which was identified by its ability to bind to DNAase I-Sepharose and to co-migrate with skeletal muscle actin and two low molecular weight polypeptides with molecular weights of 16 000 (pI 6.5) and 13 000 (pI 5.3), dominated both the Coomassie blue and [^{35}S]-methionine isoelectric focusing profiles (Fig. 3a and b). However, most of the Coomassie blue-stained proteins were detectable using [^{35}S]methionine (250

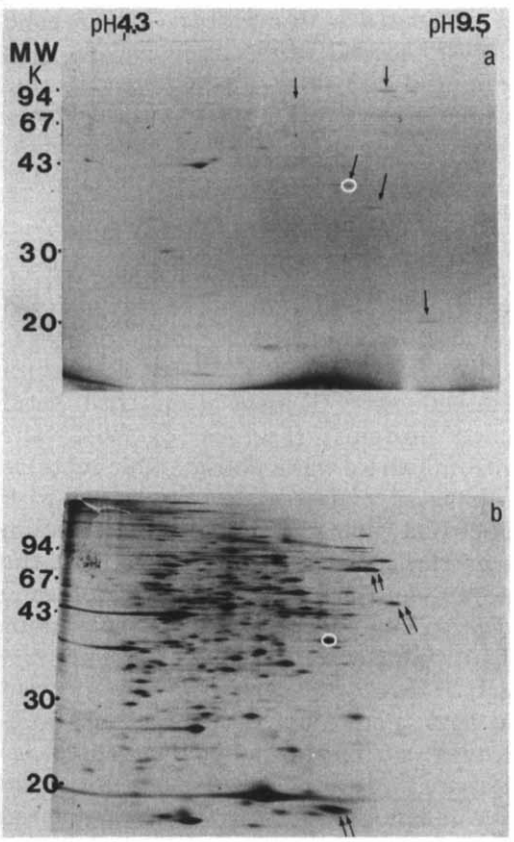

Fig. 4. Separation of [^{35}S]methionine-labeled neutrophil proteins using two-dimensional non-equilibrium pH gradient electrophoresis-SDS gels. (a) Coomassie blue-stained proteins, prominent basic proteins are indicated with the arrows; (b) autoradiograph exposed for 10 days at —70°C. Note most of the prominent Coomassie blue-stained basic proteins were not detected on the autoradiograph.

μCi/ml) for 3 h and subsequent autoradiography for 10 days. Despite the unusual intensity of these three proteins, when the autoradiograph was exposed for 42 days, more of the proteins undetected on the Coomassie blue profiles (such as No. 4, Fig. 3a—c) could be detected on the autoradiogram. One prominent protein on the Coomassie blue profile (downward arrow, Fig. 3a and b) was not detectable on any of the [^{35}S]methionine autoradiographs (Fig. 3c). One of the most acidic proteins (labeled A, Fig. 3c), detectable only after extended autoradiography, corresponded to the major surface labeled protein (see later). Prolonged exposure allowed the detection of more than 450 individual neutrophil proteins.

Analysis of the murine neutrophil proteins by non-equilibrium pH gradient electrophoresis revealed a set of basic proteins (Fig. 4a), which were not present on the isoelectric focusing gels. The slowly migrating proteins gave sharp profiles while the very basic proteins tended to streak. The most prominent basic polypeptides had apparent molecular weights of 76 000 (presumably lactoferrin; indicated by the single arrow at 76 kdaltons in Fig. 4a), 38 000 and 33 000. Two prominent polypeptides also occurred near 18 000 kilodaltons. Of these major Coomassie blue-stained basic proteins, only the one with a molecular weight of 38 000 was associated with a radioactive ^{35}S band (encircled component in Fig. 4). However, several other basic components which were labeled with [^{35}S]methionine did not stain with Coomassie blue (e.g., the components indicated by double arrows in Fig. 4b). Although prolonged exposure of the ^{35}S-labeled non-equilibrium pH gradient electrophoresis-SDS profiles revealed more labeled proteins, the basic components mentioned above (including lactoferrin) were still not detectable using autoradiography.

Analysis of external cell surface proteins

Surface-exposed proteins labeled with ^{125}I using Iodogen were analysed by two-dimensional gel electrophoresis. When radioactive iodination was catalyzed using either 5 or 50 μg Iodogen with 200 μCi ^{125}I, there was no detectable internal labeling (as determined by ^{125}I association with actin and other major Coomassie blue-stained proteins) (Fig. 5a—c). When the ^{125}I concentration was increased to 1 mCi/ml, there was a considerable increase in the number of iodinated proteins, however, ^{125}I was also associated with actin. Presumably, the extra proteins labeled at the higher iodide concentration were cytoplasmic proteins rather than extrinsic surface proteins. None of the proteins labeled in the presence of Iodogen were detectable on the Coomassie blue-stained profiles, indicating that the iodinated proteins represent minor components of the cell. The major neutrophil surface-labeled protein (86—97 kdaltons) focused near pH 4.1 (Fig. 5a). However, the use of more Iodogen or more ^{125}I (Fig. 5b and c) indicated that there may be two or three components associated with this protein. Lower concentrations of Iodogen (5 μg) (Fig. 5a and b) yielded a similar pattern of ^{125}I labeling (Fig. 5c), although less total ^{125}I was incorporated into the membrane. There was a differential labeling of some proteins at higher Iodogen concentrations. The group of four spots labeled B (Fig. 5b and c) and the ^{125}I proteins migrating close to actin contained a greater proportion of the ^{125}I label when the cells were labeled with 50 μg of Iodogen or higher ^{125}I concentrations. Analysis of the

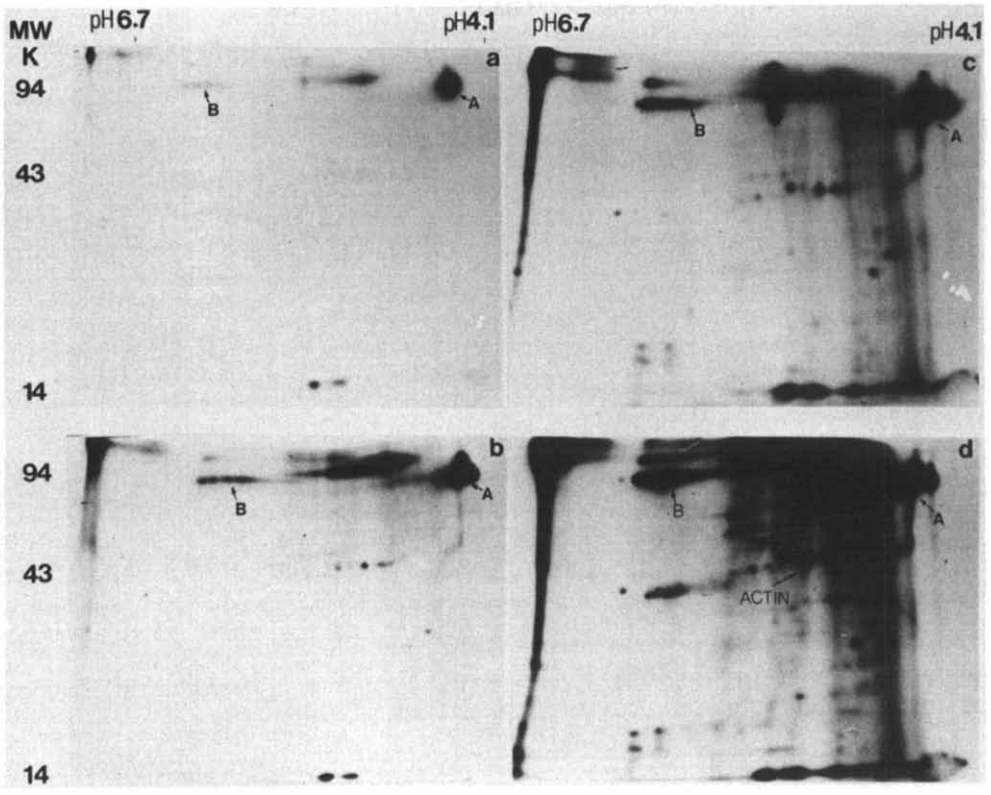

Fig. 5. ^{125}I-labeled extrinsic neutrophil proteins assayed using isoelectric focusing-SDS gels. Neutrophils were labeled using different amounts of Iodogen and $^{125}I^-$: (a) 5 μg Iodogen, 200 μCi $^{125}I^-$; (b) 5 μg Iodogen, 1 mCi $^{125}I^-$; (c) 50 μg Iodogen, 200 μCi $^{125}I^-$; and (d) 50 μg Iodogen, 1 mCi $^{125}I^-$. Autoradiographs were exposed for 10 days at −70° C using an intensifying screen.

surface-labeled neutrophil proteins using non-equilibrium pH gradient electrophoresis and SDS-polyacrylamide gels did not yield any new ^{125}I-labeled proteins — almost all of the extrinsic surface proteins must be acidic. In total, approx. 20 neutrophil proteins appear to be available for surface labeling in the presence of Iodogen.

Surface labeling of other cell types using lactoperoxidase yielded many more apparent extrinsic proteins [18]. The neutrophil surface proteins have been compared directly with the surface-labeled proteins of murine thymocytes (Fig. 6a and b). The labeling conditions (5 μg Iodogen and 1 mCi $^{125}I^-$ for neutrophils; 5 μg Iodogen and 200 μCi $^{125}I^-$ for the thymocytes) should have labeled more neutrophil proteins. However, the thymocytes appeared to have many more extrinsic proteins accessible under the conditions of labeling. No ^{125}I was associated with thymocyte actin (Fig. 6b). The pattern of labeled proteins was quite different for these cell types, only one of the proteins (apparent M_r approx. 200 000) which focused over a broad pH range between 5.5 and 4.5 appears to be similar. Many more of the ^{125}I-labeled proteins from thymocytes migrated as bands of apparently charged related proteins, presumably related because of sialic acid differences.

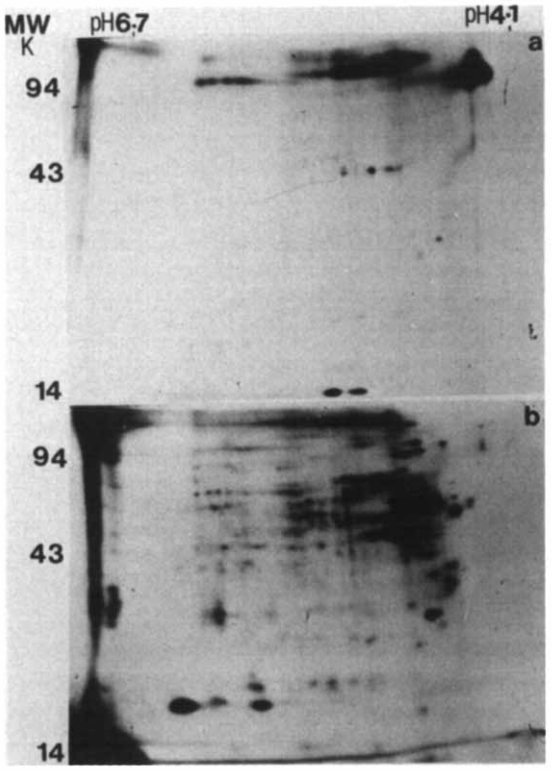

Fig. 6. ^{125}I surface-labeling patterns from neutrophils and thymocytes. (a) Neutrophils were labeled using ^{125}I (1 mCi) in the presence of 5 μg of Iodogen; (b) thymocytes were labeled using $^{125}I^-$ (200 μCi) in the presence of 5 μg of Iodogen. The autoradiographs were exposed for 10 days at −70°C using an intensifying screen.

Discussion

Analysis of developing systems and mammalian cell differentiation at a molecular level will be greatly facilitated by two-dimensional gel electrophoresis [6]. However, initial experiments have been directed more towards the understanding of inducible tumor differentiation [19] or the analysis of rather heterogeneous cell populations such as splenocytes [20]. Experience with the preparation of mammalian cells for two-dimensional analysis appears to be extremely limited and since many artifacts have been reported [6], it was interesting to examine a purified, homogeneous population [5,21] of murine neutrophils. A detailed electrophoretic map of murine neutrophil external proteins, the proteins being synthesised and the stored proteins will serve as a basis to study the development of these cells from their immature bone marrow precursors.

In comparison to similar analyses of spleen cells [20], monkey kidney cells [7] and rat hepatoma proteins [19], the Coomassie blue-stained and autoradiographed electropherograms of murine neutrophil proteins are dominated by actin under the experimental conditions used. Selective incorporation

of methionine into actin could not explain such observations, since the relative amounts of ^{35}S associated with each polypeptide did not change even when the methionine concentrations and cell densities were varied. Such a finding is not unexpected, since actin is known to comprise 10% of the neutrophil protein content and is essential for both cell motility and phagocytosis, processes essential to the functioning of the mature neutrophil.

Whilst the non-equilibrium pH gradient electrophoresis-SDS gels did not appear to resolve as many proteins, several of the cationic proteins were clearly resolved. It was interesting that the major cationic proteins did not label with [^{35}S]methionine. The possibility that these proteins do not contain methionine should be considered. Thus detailed studies with a particular protein would be best performed with several radioactively labeled amino acids. This is consistent with the synthesis and packaging of these proteins during an earlier stage of the differentiation process [22].

Some of the power of two-dimensional analysis is lost on immediate inspection of the total protein profiles. There are so many proteins that it becomes difficult to focus on regions of interest, especially with respect to differentiation. This is in part illustrated by the use of two-dimensional electrophoresis for a study of the inducible differentiation of myeloid leukemic cells [10]. In that report, 73 proteins were altered after induction of the leukemic cells with the hemopoietic regulator granulocyte-macrophage colony stimulating factor. None of these changes could be related to neutrophil development because neutrophil profiles were not available. Combining the results of some of our studies with the electrophoretic data on the leukemic cell line [10], it is possible to identify the appearance of some neutrophil specific proteins as a result of stimulation by the colony stimulating factor (e.g., the very high molecular weight protein focused at pH 5.1 appears to be typical of mature neutrophils).

As an initial attempt to identify some of the membrane proteins, neutrophils were surface labeled using ^{125}I and Iodogen. Only 20 neutrophil proteins were labeled significantly with ^{125}I and none of these appeared to correspond to any of the Coomassie blue- or [^{35}S]methionine-labeled components. The restricted labeling pattern was not due to the labeling procedure: almost 75 extrinsic thymocyte proteins were labeled using this method. Analysis of surface-labeled baby hamster kidney fibroblast clones [23] using the lactoperoxidase-glucose oxidase method also yielded a relatively simple two-dimensional electrophoretic map for the ^{125}I-labeled proteins (less than 20 major spots). Interestingly, the major labeled protein on the fibroblast surface is extremely acidic (p$I \sim 3$) with a molecular weight between 90 000 and 110 000, which is similar to the protein dominating the ^{125}I-labeled profile. Similarly, this protein appeared to focus sharply but was the only protein to spread significantly in the SDS dimension. Prolonged exposure of the autoradiographs and different labeling conditions suggest that this ^{125}I-labeled protein spot may be composed of several components. A group of ^{125}I-labeled proteins around 150 kdaltons which form a belt of five from pH 4.4 to 5.3 in the fibroblast map [23] bears a striking resemblance to the 150 kdalton belt in our ^{125}I-labeled murine neutrophil amps. These differences presumably reflect real heterogeneities in partially sialyated surface glycoproteins, rather

than artifacts during sample preparation [6] which may be the explanation of some of the band multiplicity in surface-labeled spleen cells [18].

The ^{125}I-labeled protein with an apparent molecular weight of 200 000, focusing between pH 5.5 and 4.5, was present on both neutrophils and thymocytes. This protein appears to be similar to the leukocyte common antigen described in detail for rat thymocytes [24]. Our further studies will be directed to analysis of proteins from subcellular fractions, e.g., membrane preparations. Initial two-dimensional analyses of human red cell membranes have detected over 200 'proteins' but some of the spots are almost certainly generated by the method of analysis [25]. It will be interesting to compare membranes from various cells of the same animal species to see if an integrated picture of structural and specialised proteins can be obtained.

Acknowledgments

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References

1 Chan, L.-N.L. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 1062—1066
2 Edwards, J.J., Anderson, N.G., Nance, S.L. and Anderson, N.L. (1979) Blood, 1121—1132
3 Metcalf, D. (1977) Hemopoietic Colonies; In Vitro Cloning of Normal and Leukemic Cells, Springer-Verlag, Heidelberg
4 Burgess, A.W., Camakaris, J. and Metcalf, D. (1977) J. Biol. Chem. 252, 1998—2003
5 Watt, S.M., Burgess, A.W. and Metcalf, D. (1979) J. Cell. Physiol. 100, 1—22
6 O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007—4021
7 O'Farrell, P.Z., Goodman, H.M. and O'Farrell, P.H. (1977) Cell 12, 1133—1142
8 Ichikawa, Y. (1969) J. Cell. Physiol. 74, 223—234
9 Metcalf, D. (1980) Int. J. Cancer, in the press
10 Hoffman-Liebermann, B. and Sachs, L. (1978) Cell 14, 825—834
11 Burgess, A.W. and Metcalf, D. (1977) J. Cell. Physiol. 90, 471—484
12 Bainton, D.F. and Fahrquhar, M.G. (1966) J. Cell Biol. 28, 277—301
13 De St. Groth, S.F., Webster, R.G. and Datyner, A. (1963) Biochim. Biophys. Acta 71, 377—391
14 Fraker, P.J. and Speck, J.C. (1978) Biochem. Biophys. Res. Commun. 80, 849—857
15 O'Farrell, P.Z. and O'Farrell, P.H. (1977) Methods Cell Biol. 16, 407—420
16 Laemmli, U.K. (1970) Nature 227, 680—685
17 Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83—88
18 Ledbetter, J.A. and Herzenberg, L.A. (1979) Immunol. Rev. 47, 63—90
19 Ivarie, R.D. and O'Farrell, P.H. (1978) Cell 13, 41—56
20 Jones, P. (1977) J. Exp. Med. 146, 1261—1279
21 Potter, T.A., Watt, S.M., Burgess, A.W. and McKenzie, I.F.C. (1979) Immunogenetics 8, 461—473
22 Bainton, D.F., Ullyot, J.L. and Farquhar, M.G. (1971) J. Exp. Med. 134, 907—934
23 Pena, S.D.J., Mills, G. and Hughes, R.C. (1979) Biochim. Biophys. Acta 550, 100—109
24 Fabre, J.W. and Williams, A.F. (1977) Transplantation 23, 349—359
25 Rubin, R.W. and Milikowski, C. (1978) Biochim. Biophys. Acta 509, 100—110