activities) are generally not found on short synthetic peptides. Unlike the repeat region of *P. falciparum*, the *P. berghei* CS protein contains variable tandem repeats consisting of three different octapeptides and a long stretch of dipeptides (*8*). We used the consensus octapeptide for synthetic peptide immunization, but it is possible that other epitopes may be important in protection. The recombinant construct pB1tet$_{32}$ included the entire repeat region sequence as well as flanking nonrepeat regions, suggesting that if T-cell epitopes involved in the induction of cellular immunity were included they were ineffective because of inappropriate conformation or antigen presentation. Attenuated sporozoites must be intact and administered intravenously to be efficacious (*21*), suggesting that targeting of the organism to a particular cell and subsequent processing and presentation of antigen in association with histocompatibility molecules may be important. From studies in vitro it appears that sporozoites shed CS protein onto the surface of hepatoma cells during invasion and that developing exoerythrocytic forms express epitopes recognized by mAbs against sporozoites (*22*). It is possible that CS protein or other non-CS antigens associated with these developing exoerythrocytic forms elicit responses mediated by natural killer cells, cytotoxic T cells, or cytokines. However, the antigens, specific cellular effector mechanisms, and their targets have not been identified. By using the *P. berghei* model it should be possible to develop subunit vaccines capable of inducing these cell-mediated responses and to apply these findings to the development of sporozoite vaccines against human malaria.

REFERENCES AND NOTES


1. R. S. Nussenzweig, J. Vanderberg, H. Most, *Mil. Med.* **134**, 1176 (1969). D. F. Clyde *et al.*, *Am. J. Trop. Med. Hyg.* **24**, 397 (1975); K. H. Rieckmann, R. L. Beaudoin, J. S. Cassells, K. W. Sell, *Bull. WHO* **57** (suppl. 1), 261 (1979).
2. W. T. Hockmeyer and J. B. Dame, *Adv. Exp. Med. Biol.* **185**, 233 (1985).
3. J. L. Weber and W. T. Hockmeyer, *Mol. Biochem. Parasitol.* **15**, 305 (1985); F. Zavala *et al.*, *J. Immunol.* **135**, 2790 (1985).
4. J. B. Dame *et al.*, *Science* **225**, 593 (1984); V. Enea *et al.*, *ibid.*, p. 628.
5. W. R. Ballou *et al.*, *ibid.* **228**, 996 (1985); F. Zavala *et al.*, *ibid.*, p. 1436.
6. P. Potocnjak, N. Yoshida, R. S. Nussenzweig, V. Nussenzweig, *J. Exp. Med.* **151**, 1504 (1980).
7. S. L. Hoffman *et al.*, *N. Engl. J. Med.* **315**, 601 (1986).
8. J. L. Weber *et al.*, *Exp. Parasitol.*, in press; D. J. Eichinger *et al.*, *Mol. Cell. Biol.* **6**, 3965 (1986).
9. Peptides were synthesized by the solid phase technique of R. B. Merrifield *et al.* [*Annu. Rev. Biochem.* **39**, 841 (1970)] with a Beckman 990 peptide synthesizer. Purity was confirmed by reversed-phase high-performance liquid chromatography and amino acid composition analysis. Equal amounts of peptide and KLH were combined (1:1 w/w) in 1% glutaraldehyde in phosphate-buffered saline (PBS) and stirred for 3 hours at room temperature, dialyzed extensively against PBS, and lyophilized to dryness.
10. A Taq I fragment encoding 82% of the complete *P. berghei* CS gene was modified to prepare clone pB1-6 (J. L. Weber *et al.*, *Exp. Parasitol.*, in press), digested with Taq I, treated with DNA polymerase I (Klenow fragment) and deoxycytidine triphosphate to partially fill in the 5′ overhang, and treated with mung bean nuclease to remove the remaining single base overhang. The DNA was digested with Ssp I and a 763–base pair fragment was isolated and ligated into Bam HI–digested Klenow-treated pAS1 that had been modified by deleting a 14–base pair Bam II fragment in the tet$^r$ regions as described [Young *et al.* (*11*)]. The resulting construct encodes 254 amino acids and extends from Taq I, which begins 40 amino acids 5′ of the first octapeptide repeat, includes all of region I (*4*), the central repeat units, region II (*4*), and ends in the hydrophobic anchor sequence 18 amino acids 5′ of the carboxyl-terminal asparagine residue of the complete protein. This polypeptide is fused to 32 amino acids derived from the tet$^r$ region of the expression vector. The construct was expressed in *E. coli* under the control of the $P_L$ promoter as described [J. F. Young, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 6105 (1983)]. The *P. berghei* fusion protein extracted from induced *E. coli* with 0.1% (w/v) sodium deoxycholate was present at approximately 5 to 10% of the total protein precipitated with 20 to 40% ammonium sulfate.
11. J. F. Young *et al.*, *Science* **228**, 958 (1985).
12. Antigens were emulsified 1:1 in complete Freund's adjuvant [CFA], (Gibco) for primary immunization and in incomplete Freund's adjuvant (IFA) for subsequent doses, and injected intramuscularly and subcutaneously. CS construct pB1tet$_{32}$ was adsorbed to aluminum hydroxide gel in 0.9*N* saline by end-over-end rotation at 4°C for 16 hours. Each 0.25-ml dose contained 0.5 mg of $Al^{3+}$.
13. Sporozoites of the NK65 strain of *P. berghei* were harvested in Medium 199 from *Anopheles stephensi* approximately 14 days after the mosquitoes had fed on infected Syrian hamsters. Sporozoites used for immunization received 8000 R from a $^{60}$Co source (Gamma Cell 220) at 4°C.
14. The ELISA, ISI, and CSP assays were performed under blind conditions on triplicate serum samples as described by Young (*11*) with the following modifications: (i) the capture antigen for ELISA was (D-16-N)-BSA, pB1tet$_{32}$, or *P. berghei* sporozoites (NK65 strain); (ii) for the ISI and CSP assays *P. berghei* sporozoites were used.
15. G. L. Spitalny, C. Rivera-Ortiz, R. S. Nussenzweig, *Exp. Parasitol.* **40**, 179 (1976).
16. Pooled serum samples from mice immunized with γ-sporozoites (D-16-N), pB1tet$_{32}$, and from normal controls (20 mice per group), and mAb 3.28.1 ascites were affinity-purified by protein A–Sepharose 4B chromatography (*7*). The mAb 3.28.1 was derived from spleen cells of mice immunized with γ-sporozoites as described elsewhere [H. D. Danforth, G. H. Campbell, M. F. Leef, R. L. Beaudoin, *J. Parasitol.* **68**, 1029 (1982)]. The protein concentration of the final product was determined spectrophotometrically at a wavelength of 280 nm.
17. D. H. Chen, R. E. Tigelaar, F. I. Weinbaum, *J. Immunol.* **118**, 1322 (1977).
18. J. P. Verhave, G. T. Strickland, H. A. Jaffe, A. Ahmed, *ibid.* **121**, 1031 (1978).
19. J. Ipsen, *N. Engl. J. Med.* **251**, 459 (1954).
20. T. Dyrberg and M. B. A. Oldstone, *J. Exp. Med.* **164**, 1344 (1986).
21. G. L. Spitalny and R. S. Nussenzweig, *Proc. Helm. Soc. Wash.* **39**, 506 (1972).
22. M. Hollingdale, P. Leland, J. Leef, A. L. Schwartz, *Am. J. Trop. Med. Hyg.* **32**, 685 (1983); A. Szarfman *et al.*, in preparation.


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# Glucocorticoid Receptor–Like Antigen in Lymphoma Cell Membranes: Correlation to Cell Lysis


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**S-49 mouse lymphoma cells undergo lysis when treated with glucocorticoids; the mechanism of this effect is not understood. A protein was detected in the plasma membrane of these cells by means of direct immunofluorescent labeling with a monoclonal antibody to the soluble glucocorticoid receptor. Cellular heterogeneity in the content of this glucocorticoid receptor–like molecule was evident. By immunoadsorption to antibody-coated tissue culture plates, the cells were separated into populations positive (100%) and depleted (38%) for this membrane antigen. Gel electrophoresis, specific immunoblot, and autoradiographic (binding of [$^3$H]dexamethasone mesylate) analysis of the membrane proteins from the membrane antigen–positive group revealed multiple protein bands ranging in size from 85 to 145 kilodaltons. Furthermore, comparison of the glucocorticoid sensitivity of these groups of cells showed complete lysis of the membrane antigen–positive cells and only partial lysis of the antigen-deficient group, which suggests that the lysis response of cells to glucocorticoids is mediated by a glucocorticoid receptor–like molecule located in the plasma membrane.**


GLUCOCORTICOID HORMONES ELICIT a series of cellular responses in lymphoid tissues that ultimately result in cytolysis, a property that allows important practical applications of these steroids in the treatment of certain leukemias and lymphomas (*1, 2*). Although the exact mechanism of this induced cell lysis is obscure, the effect is known to be mediated by the glucocorticoid receptor (GR) (*3*). In some patients with various blood dyscrasias, high numbers of lymphoid cell receptor sites have been correlated with good clinical response to gluco-


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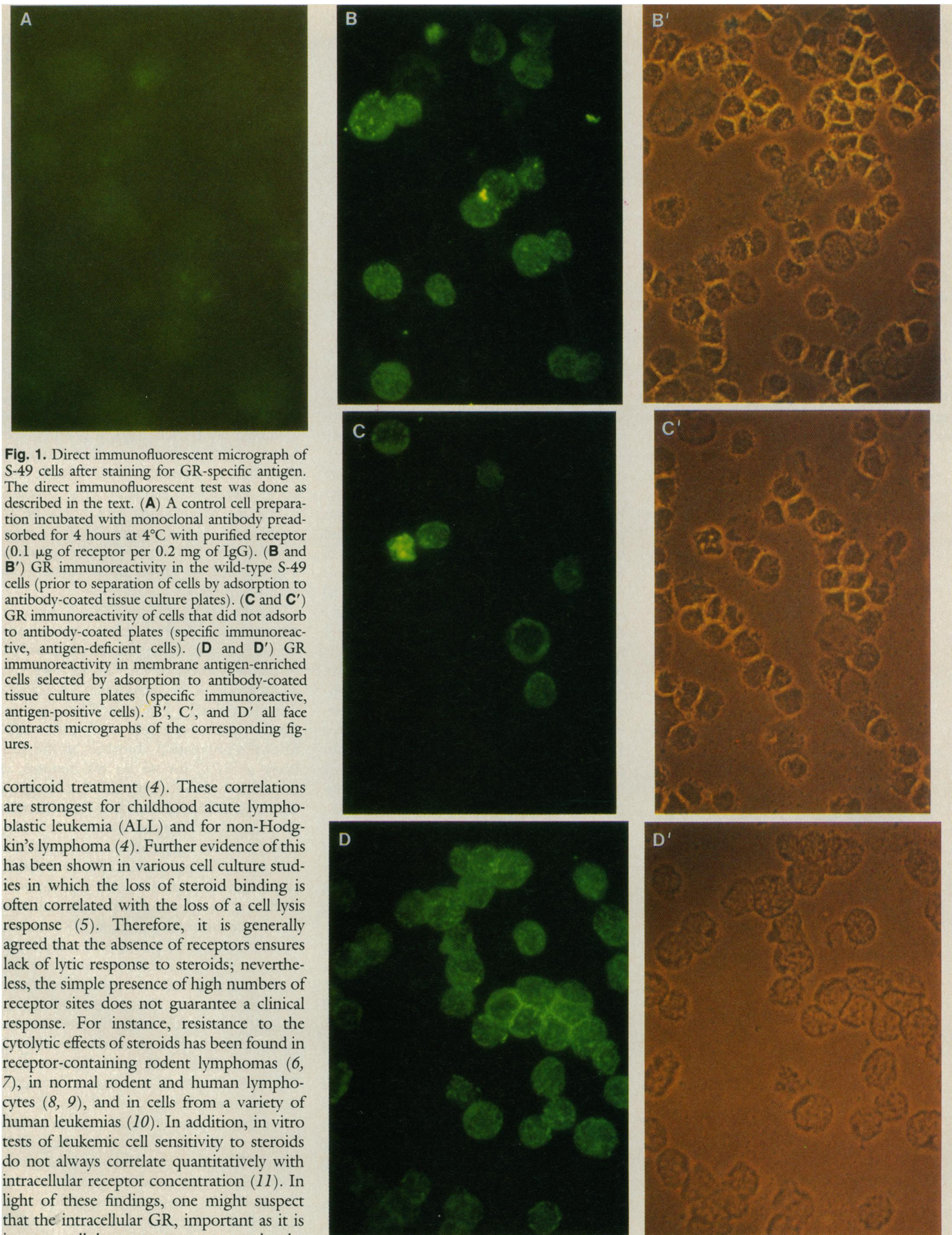


**Fig. 1.** Direct immunofluorescent micrograph of S-49 cells after staining for GR-specific antigen. The direct immunofluorescent test was done as described in the text. (**A**) A control cell preparation incubated with monoclonal antibody preadsorbed for 4 hours at 4°C with purified receptor (0.1 μg of receptor per 0.2 mg of IgG). (**B** and **B′**) GR immunoreactivity in the wild-type S-49 cells (prior to separation of cells by adsorption to antibody-coated tissue culture plates). (**C** and **C′**) GR immunoreactivity of cells that did not adsorb to antibody-coated plates (specific immunoreactive, antigen-deficient cells). (**D** and **D′**) GR immunoreactivity in membrane antigen-enriched cells selected by adsorption to antibody-coated tissue culture plates (specific immunoreactive, antigen-positive cells). B′, C′, and D′ all face contracts micrographs of the corresponding figures.

corticoid treatment (*4*). These correlations are strongest for childhood acute lymphoblastic leukemia (ALL) and for non-Hodgkin's lymphoma (*4*). Further evidence of this has been shown in various cell culture studies in which the loss of steroid binding is often correlated with the loss of a cell lysis response (*5*). Therefore, it is generally agreed that the absence of receptors ensures lack of lytic response to steroids; nevertheless, the simple presence of high numbers of receptor sites does not guarantee a clinical response. For instance, resistance to the cytolytic effects of steroids has been found in receptor-containing rodent lymphomas (*6, 7*), in normal rodent and human lymphocytes (*8, 9*), and in cells from a variety of human leukemias (*10*). In addition, in vitro tests of leukemic cell sensitivity to steroids do not always correlate quantitatively with intracellular receptor concentration (*11*). In light of these findings, one might suspect that the intracellular GR, important as it is in many cellular responses, may not be the

only pathway by which the lytic effect of glucocorticoids is mediated.

Perhaps one area that deserves further study is the mechanism by which steroids gain cell entry. The plasma membrane could be involved in steroid resistance, much as the restriction of drug transport by the plasma membrane plays an important role in the mechanism of drug resistance (*12*). It has generally been shown that steroids by virtue of their small size and lipophilic nature enter cells by passive diffusion through the plasma membrane (*13*). Nevertheless, some recent studies support the role of a saturable membrane-mediated entry of several steroid hormones in certain cell types (*14, 15*). Huet-Minkowiski *et al.* recently demonstrated that plasma membrane permeability can be a rate-limiting step in steroid hormone action and is the basis for dexamethasone resistance of a mouse thymoma cell line variant (*16*). In addition, the same group has reported that adenosine 3′,5′-monophosphate (cAMP)–dependent protein kinase plays a role in regulating GR function (*17*). Furthermore, a more recent study by Allera and Rao has shown that the uptake of corticosterone by the plasma membrane vesicle is saturable and reversible (*14*). Several investigators pointed to the possibility that the steroid receptors, including GR, are localized in the membrane (*18–25*) though, for the most part, direct and convincing evidence has been lacking.

In this report, I present (i) immunological evidence that GR or an antigenically related protein or proteins are located in the plasma membrane in S-49 lymphoma cells and (ii) evidence that this plasma membrane protein may be involved in glucocorticoid-mediated lymphocytolysis.

Glucocorticoid lysis–sensitive, wild-type S-49 mouse lymphoma cells (*17*) were grown as suspension aggregates in RPMI 1640 medium (Irvine Scientific) supplemented with 10% fetal calf serum in a 37°C humidified atmosphere containing 5% $CO_2$.

Immunocytochemical localization of the GR was carried out by direct immunofluorescence (*26*) in which the purified monoclonal antibody to the GR (*27*) was conjugated with fluorescein isothiocyanate (FITC). The direct fluorescent antibody-labeling approach used in these studies was preferred to an indirect fluorescent detection method previously used by others because of its superior specificity (*28, 29*). S-49 cells ($\sim 10^5$ cells) were centrifuged at 900*g* for 10 minutes onto a cover slide, fixed by air drying, and incubated with antibody for 1 hour at 37°C. After the incubation, the preparations (on seven slides, where an average of five fields per slide were examined) were washed three times with phosphate-buffered saline (PBS) and examined with a fluorescent microscope (Zeiss, Model Orthoplan). The results show that only 53.0 ± 9.5% (SEM) of the S-49 cells contained specific immunofluorescing GR. Heterogeneity in immunostaining for steroid receptors has been observed in several tissues consisting of the same (*30, 31*) or different (*28, 29, 32*) cell types. However, my data indicate heterogeneity of GR in a cloned cell population. The GR-specific fluorescence was diffuse and bright both in the cytoplasmic and nuclear compartments as well as in the periphery of the cells, which was notably heavily stained (Fig. 1, B and B′). The specificity of the antibody was confirmed both by staining of S-49 cells with fluorescent antibody previously adsorbed with purified GR preparation and with nonspecific FITC-conjugated rabbit antibody to rat immunoglobulin G (IgG) showing, in both cases, negligible cellular fluorescence (Fig. 1A). From the marked intensity and punctate appearance of peri-membrane-specific immunofluorescence in positive cells, I suspected that the glucocorticoid receptor or an antigenically similar molecule was located in patches on the plasma membrane.

I took advantage of this predicted surface location of GR to isolate and grow separately the GR-positive and the GR-negative cells. Tissue culture plates were coated with monoclonal antibodies, and cells were incubated in these dishes for 1 hour at 37°C in a humidified incubator containing 5% $CO_2$. After the incubation, nonadhering cells were washed off the plates, collected, and allowed to replicate separately. The adhering cells were rinsed with several changes of PBS, removed by repeated pipetting, and grown as above. Immunocytochemical staining of the nonadhering cells (on seven slides, with an average of five fields examined per slide) revealed specific immunofluorescence in 38.9 ± 2.5% of the cell population (Fig. 1, C and C′). In contrast, cells that adhered to the dish showed 100% bright-specific fluorescence (Fig. 1, D and D′), indicating complete enrichment of GR-positive cells based on the ability of a surface antigen to bind them to immobilized GR-specific antibody. Fluorescence-labeled cells contaminating the nonadhering group may be accounted for by the requirement for a minimum number of receptors on the surface of a given cell to effect an attachment via antibody binding.

Results from both air-dried cell immunofluorescence and whole cell immunoadsorption experiments strengthened the possibility that a portion of the GR in S-49 cells could reside in the membrane. To examine this more closely, I incubated live (not air-dried) intact S-49 cells ($\sim 5 \times 10^5$ cells) from the population absorbed by the antibody at 37°C for 45 to 60 minutes, as mentioned above, with FITC-conjugated antibody. After the reaction, the cells were centrifuged at 600*g* and washed three times with PBS. The cell pellet was resuspended in 100 μl of 20% glycerol in PBS, dropped on slides, and covered with cover slips. In order to flatten the spherical S-49 cells and show clearly the fluorescent granules on the membrane surface, mild pressure was applied to some cover slips. I then examined the cells under the ultraviolet microscope for membrane fluorescence. The results showed large granules of specific bright fluorescence exclusively on the plasma membranes (Fig. 2, A and B). Unlike the results illustrated in Fig. 1, B, C, and D, cytoplasmic or nuclear GR could not be detected in this case because the integrity of plasma membrane was preserved by the live cell–labeling technique. Therefore, antibody molecules only had access to the membrane receptor. Furthermore, the presence of specific plasma membrane antigen was confirmed by whole cell radioimmunoassays (*33*).

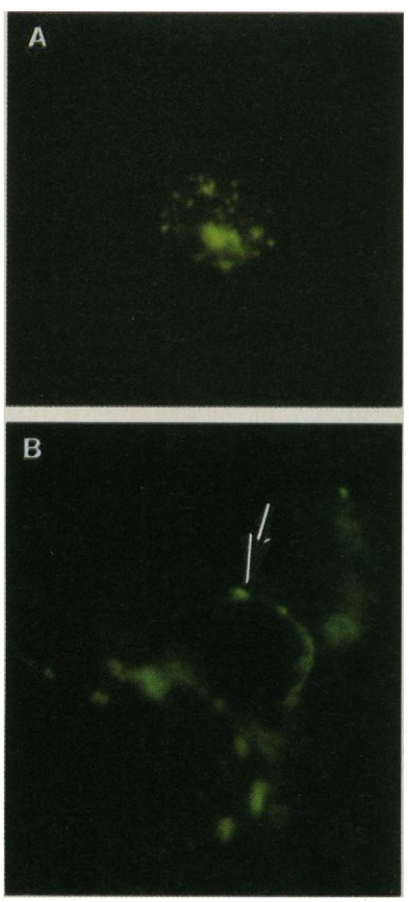


**Fig. 2.** (**A** and **B**) GR immunoreactivity shown in the periplasma membrane of live S-49 cells flattened (pressure applied to the cover slip) and spherical, respectively (see text for further explanation). All magnifications are ×1000.

These experiments also suggested that patching and capping of membrane protein antigens could be occurring (see Fig. 2A and 2B, arrow). In order to test this possibility more systematically, live cell immunofluorescent labeling was carried out for various times of incubation ranging from 15 to 60 minutes. At 15 minutes, small granules (about 0.2 to 0.5 $\mu M$ in diameter) of specific fluorescence were apparent at multiple sites on the periphery of the plasma membrane (Fig. 3A). Thirty minutes after the start of the incubation, diffuse but larger granules of fluorescence began to appear at one side of the cell membrane (Fig. 3B). At 60 minutes, large granules (0.5 to 1.0 $\mu M$) of specific fluorescence were often detectable as a cap at one pole of the cells (Fig. 3C).

Further verification that the immunoreactive membrane protein was a glucocorticoid receptor was accomplished by analysis of partially purified membrane preparations. GR labeling in whole cells was accomplished by incubating $2 \times 10^8$ cells for 2 hours on ice in $2 \times 10^{-8} M$ [$^3$H]dexamethasone 21-mesylate (*25*). This was done both to stabilize the receptor during membrane preparation and for affinity-labeling studies. The antibody-adsorbed cells were lysed by repeated freeze-thaw cycles. A nuclear pellet was obtained by low-speed centrifugation (600*g* for 10 minutes) of the lysed cell preparation. The supernatant was further centrifuged at 15,000*g* for 10 minutes to pellet mitochondria and lysosomes. The remaining supernatant was centrifuged at 120,000*g* for 2 hours, yielding a supernatant (cytosol) and membrane-containing pellet (high-speed particulate) (*19*). Duplicate samples of varying amounts of protein from nuclear and cytoplasmic preparations and the membrane-containing high-speed particulate fraction (concentrated by immunoprecipitation) were resolved by 7.5% SDS–polyacrylamide gel electrophoresis (PAGE). The protein bands were electrophoretically transferred onto a nitrocellulose filter, and immunoblot and autoradiographic analyses were performed (*27*) (Fig. 4). The nuclear and cytoplasmic preparations contained two identical immunoreactive bands of 97 and 70 kD (Fig. 4: lanes A and B, nucleus; lanes C and D, cytoplasmic). The high-speed particulate (membrane-containing) preparation that had been immunoprecipitated also contained the 97-kD band (Fig. 4, lane E). In addition, this preparation also contained five other immunoreactive bands, four of which were larger than the nuclear or cytoplasmic receptors and ranged in size from 112 to 145 kD; however, the 70-kD species present in both the cytoplasmic and nuclear samples was not present in the particulate fraction. This smaller protein is most likely a degradation product of the 97-kD monomeric form of the receptor. It was probably absent from the immunoprecipitated membrane preparation because bound antibodies are known to protect antigenic sites of some proteins from the action of proteases (*34*). The two immunoreactive smaller bands detectable in lane E (56 and 24 kD) were heavy and light immunoglobulin chains. Lane F is an autoradiogram of lane E showing that at least three of the five immunoreactive protein bands from the plasma membrane, with $M_r$ 97, 112, and 115 kD, contained steroid-binding sites. The tritiated steroid bound to these sites could be displaced with nonradiolabeled dexamethasone (100-fold excess) included during the labeling period. Lane G contains a membrane preparation that was immunoprecipitated with mouse γ-globulin not directed toward GR, and lane H contains the membrane preparation treated only with antibody to mouse IgG but not monoclonal antibody to GR. These controls show the specificity of the monoclonal antibody to the membrane protein or proteins, since neither the control mouse IgG nor the secondary antibody (antibody to mouse IgG used in the immunoassay) produced a positive reaction when used alone.

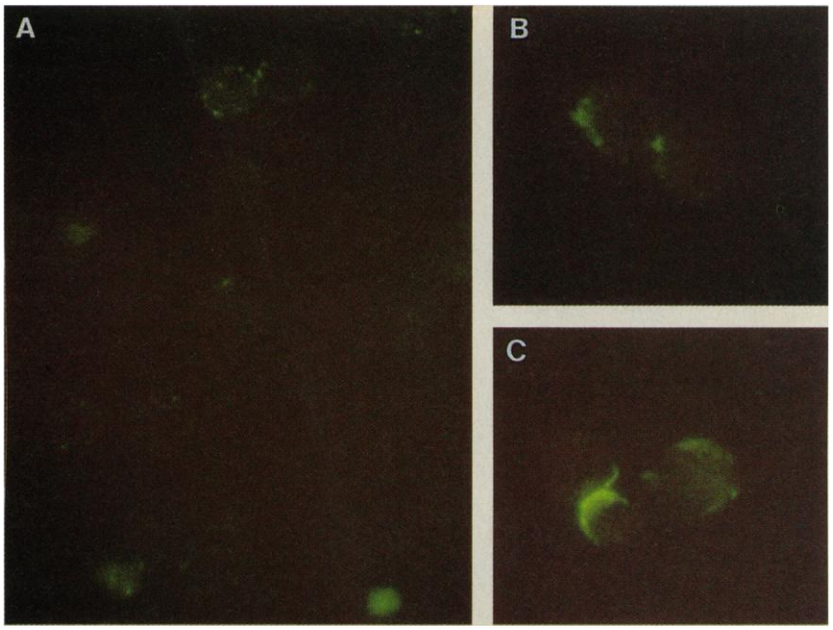


**Fig. 3.** Direct immunofluorescent micrograph of live (not air-dried) S-49 cells after staining for plasma membrane–specific antigen. (**A**) Cells after incubation with antibody for 15 minutes (magnification ×160). (**B**) After incubation with antibody for 30 minutes (magnification ×200). (**C**) After incubation with antibody for 60 minutes (magnification ×160).

These results confirm that the GR, or a GR-like protein, located in the lymphoma cell membrane consists of several forms of the receptor, with as many as four being larger than the monomeric form of the receptor. The differences in size could well be attributed to a post-translational modification (for example, glycosylation, phosphorylation, methylation) of the protein (*35*) or to an additional peptide component that allows the protein to be localized in the membrane. Phosphorylation of cytoplasmic and nuclear receptors for steroid hormones have been described, but phosphorylation of membrane-associated receptors has not been demonstrated. Singh and Moudgil (*35*), working with rat liver GR, reported the presence of four phosphorylated protein bands of 45, 90, 116, and 190 kD. Although the 90- and 45-kD proteins were reported to be the monomeric form of the receptor and its degradation product, respectively, the 116- and 190-kD proteins were regarded as either minor protein contaminants or precursors of the monomeric form of GR.

Finally, the presence of the GR-like protein in the membrane appears to be correlated with the ability of glucocorticoids to induce a lytic response in these cells, thus suggesting a possible functional role for this membrane form of the receptor. Cells other than the S-49 line—the GR-rich mouse pituitary tumor (AtT-20) (*36*) and fibroblastic L-929 (*37*) cell lines—were checked for the presence of membrane-bound GR. Neither of these cell lines showed significant membrane fluorescence, which suggests that not all receptor-positive cells bear the membrane GR. In addition, neither of these cells are lysed by glucocorticoids.

I then tested whether the presence of the GR-like membrane molecule could be di-

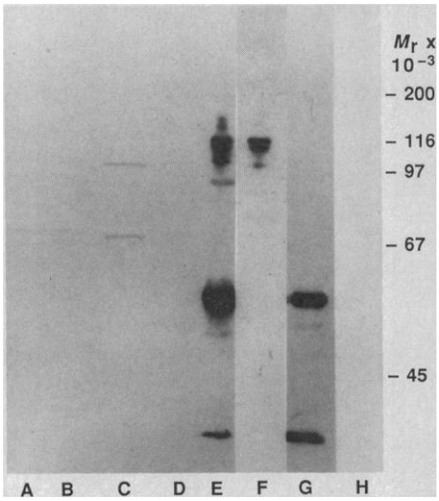


**Fig. 4.** Immunoblot and autoradiographic analysis of the nuclear and cytosolic GR as well as plasma membrane protein from specific membrane antigen–enriched cells, as described in the text. (**A** and **B**) Nuclear preparation containing 100 and 50 μg of total protein, respectively. (**C** and **D**) Cytosolic preparation containing 100 and 50 μg of total protein, respectively. (**E**) Immunoprecipitate of membrane containing particulate fraction with monoclonal antibody to GR. (**F**) Autoradiogram of lane E. (**G**) Control membrane preparation immunoprecipitated with nonspecific mouse IgG and (**H**) control membrane preparation without monoclonal antibody.

rectly correlated to the cell lysis effect of glucocorticoids. Glucocorticoid sensitivity studies were carried out by growing the three groups of cells (adsorbed, unadsorbed, and wild-type nonseparated S-49 cells) in the presence and absence of $10^{-6}M$ dexamethasone (*17*). The results from a representative experiment in which cell viability was monitored daily by the trypan blue dye exclusion method are shown in Fig. 5. The untreated cells, as expected, continued to grow logarithmically. On the other hand, 4 days after exposure to dexamethasone, 97% of the initial cell population from the antibody-adsorbed group was lysed. In the unadsorbed group, 58% of cell lysis occurred during the first half of the experiment, whereas moderate cell growth was observed (9%) during the second half. Unlike the adsorbed cells, these cells gradually overcame the effect of dexamethasone and eventually attained normal growth, possibly through growth of nonsensitive cells. The wild-type S-49 line was 88% lysed during the first 2 days and the amount of lysis did not change significantly during the remaining 2 days. Thus, there is a correlation between cell lysis and the presence of immunoreactive plasma membrane GR-like antigen. Results from the fluorescent antibody studies indicate, however, that only 39% of the unadsorbed cells contained specific membrane fluorescence, whereas 58% were lysed by glucocorticoid. The discrepancy between these findings suggests that additional factors may be involved in the mechanism of glucocorticoid-induced cell lysis, perhaps factors that are secreted into the media by steroid-sensitive cells.

The question of the cellular distribution of steroid hormone receptors has recently been actively debated. In the past, results from cell fractionation experiments led us to believe that unoccupied steroid hormone receptors reside in the cytoplasm of the target cell (*38*). According to this model, steroid hormones entering cells from the circulation bind to specific cytoplasmic receptors with high affinity, and formation of this steroid-receptor complex mediates a change in the receptor, which causes translocation into the nucleus and binding with high affinity to DNA and chromatin. The recent availability of monoclonal antibodies to the estrogen receptor has allowed its study by immunocytochemical techniques at the light microscope level (*28*). With this reagent, the receptor was found exclusively in the nucleus both in the presence and absence of hormones, and similar studies conducted with progesterone receptor corroborated these results (*30*). GR studies, so far, agree with the conventional model, that is, the receptor is both in the cytoplasm and nucleus, depending on whether hormone is bound to it (*29, 39, 40*). However, direct FITC-labeled monoclonal antibodies to this receptor (such as those used in this study) have not been used to examine this question.

Steroid-binding sites have been demonstrated on plasma membranes of cell from tissues, such as pituitary (*20*), uterine (*21*), and synaptic plasma membrane of rat brain tissue (*41*). Binding experiments, carried out more recently, concluded that the rat pituitary and liver cells plasma membrane showed specific receptor-binding sites for [$^3$H]estradiol (*14*) and [$^3$H]corticosterone (*14*). In the latter case, plasma membrane uptake of corticosteroid is saturable and seems to consist of multiple uptake systems, and thus does not follow normal saturation kinetics (*13, 42*). In all these instances, the function of the membrane-binding site is yet to be directly determined.

Peptide hormonal regulation of adenylate cyclase is usually membrane receptor–mediated, but some steroid hormones have also been reported to be involved in the regulation of cAMP-dependent protein kinase activity (*22, 23*). One well-characterized example of this steroid hormone effect is the work of Sadler and Maller, which demonstrated a correlation between progesterone inhibition of adenylate cyclase activity and an [$^3$H]R5020 affinity–labeled progesterone receptor (110 kD) in frog oocyte plasma

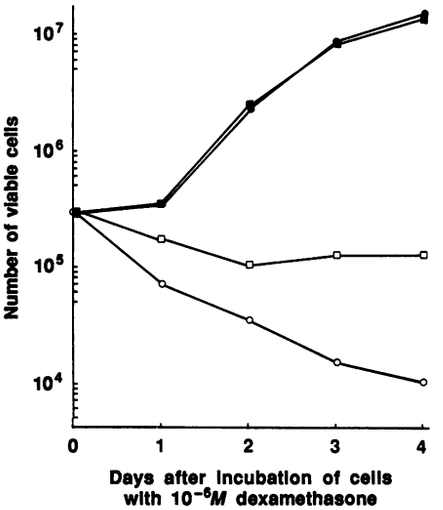


**Fig. 5.** Glucocorticoid sensitivity assay of S-49 cells. Cell viability was monitored as described in the text and expressed in $\log_{10}$ of cell number, and this result is a representative of three different experiments. (○) Dexamethasone-treated cells from specific membrane antigen–enriched group; (□) dexamethasone-treated cells from specific membrane antigen–deficient group; (●) negative control, cells from specific membrane antigen–enriched group grown in dexamethasone-free media; (■) negative control, cells from specific membrane antigen–deficient group grown in dexamethasone-free media.

membrane (*24*). Another example is a recent report from the Bourgeois laboratory showing that cAMP-dependent protein kinase plays a role in regulating GR function (*17*).

In summary, my data indicate that S-49 mouse lymphoma cell membranes contain, in addition to the 97-kD GR, larger GRs, or antigenically related proteins. This was demonstrated by direct fluorescent labeling with a monoclonal antibody to the GR and purification of cells containing this antigen by adsorption of live cells via their membrane receptors to tissue culture plates coated with antibody to GR. These selected cells were 100% membrane receptor–positive by direct fluorescent antibody analysis and were maximally responsive to the lysis effects of glucocorticoids. In addition, cell lines that contain plentiful GR but have no GR-like membrane antigen are not lysed by glucocorticoids. Reasons previously established for GR resistance of T-lymphoid cells include GR mutations as judged by their inability to bind ligand (*43, 44*) and possible DNA methylation of a "lysis gene" (*45*). My study reveals that the lysis response might additionally be mediated by the plasma membrane GR-like molecule.

## REFERENCES AND NOTES

1. H. N. Claman, *N. Engl. J. Med.* **287**, 388 (1972).
2. A. Goldin, *Cancer Chemother. Rep.* **55**, 309 (1971).
3. J. D. Baxter and A. W. Harris, *Transplant. Proc.* **7**, 55 (1975).

4. J. Stevens and Y.-W. Stevens, in *Hormonally Responsive Tumors*, V. P. Hollander, Ed. (Academic Press, Orlando, FL, 1985), pp. 156–182.
5. E. B. Thompson, J. M. Harmon, M. R. Norman, T. J. Schmidt, in *Hormones and Cancer*, S. Iacobelli *et al.*, Eds. (Raven, New York, 1980), pp. 89–98.
6. N. Kaiser, R. J. Milholland, F. Rosen, *Cancer Res.* **34**, 621 (1974).
7. M. L. Nicolson and D. A. Young, *ibid.* **38**, 3673 (1978).
8. M. Lippman and R. Barr, *J. Immunol.* **118**, 1977 (1977).
9. K. A. Smith, G. R. Crabtree, S. J. Kennedy, A. U. Munck, *Nature (London)* **267**, 523 (1977).
10. E. B. Thompson, *Cancer Treat. Rep.* **63**, 189 (1979).
11. G. Crabtree, K. Smith, A. Munck, *Cancer Res.* **38**, 4268 (1978).
12. D. Garman and M. S. Center, *Biochem. Biophys. Res. Commun.* **105**, 157 (1982).
13. E. J. Peck, J. Burgner, J. Clark, *Biochemistry* **12**, 4596 (1973).
14. A. Allera and G. S. Rao, *Adv. Exp. Med. Biol.* **196**, 53 (1986).
15. D. Bression, M. Michard, M. L. Dafniet, P. Pagesy, F. Paillon, *Endocrinology* **119**, 1048 (1986).
16. M. Huet-Minkowiski, J. C. Gasson, S. Bourgeois, *Cancer Res.* **41**, 4540 (1981).
17. D. J. Gruol, N. F. Campbell, S. Bourgeois, *J. Biol. Chem.* **261**, 4909 (1986).
18. C. M. Szego and R. J. Pietras, in *Biochemical Action of Hormones*, G. Litwack, Ed. (Academic Press, New York, 1981), vol. 8, chap. 6, pp. 307–463.
19. M. E. Fant, R. D. Harbison, R. W. Harrison, *J. Biol. Chem.* **254**, 6218 (1979).
20. T. Suyemitsu and H. Terrayame, *Endocrinology* **96**, 1499 (1975).
21. R. J. Pietras and C. M. Szego, *J. Steroid Biochem.* **11**, 1477 (1979).
22. G. S. Johnson and C. J. Jaworski, *Mol. Pharmacol.* **23**, 648 (1986).
23. M. L. Elks, V. C. Manganiello, M. Vaughan, *J. Biol. Chem.* **258**, 8582 (1983).
24. S. E. Sadler and J. L. Maller, *ibid.* **257**, 355 (1982).
25. S. S. Simons, Jr., M. Pons, D. F. Johnson, *J. Org. Chem.* **45**, 3084 (1980).
26. E. Lazarides and K. Weber, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2268 (1974).
27. B. Gametchu and R. W. Harrison, *Endocrinology* **114**, 274 (1984).
28. W. J. King and G. L. Greene, *Nature (London)* **307**, 745 (1984).
29. T. Antakley and H. J. Eisen, *Endocrinology* **115**, 1984 (1984).
30. M. Perrot-Applanat, F. Logeat, M. T. Groyer-Pickard, E. Milgrem, *ibid.* **116**, 1473 (1985).
31. H. S. Pouslen, *Eur. J. Cancer* **17**, 1495 (1981).
32. W. A. Knight, R. B. Livingston, E. J. Gregory, W. L. McGuire, *Cancer Res.* **37**, 4669 (1977).
33. B. Gametchu *et al.*, in preparation.
34. R. R. Porter, *Biochem. J.* **46**, 479 (1950).
35. V. B. Singh and V. K. Moudgil, *J. Biol. Chem.* **260**, 3684 (1985).
36. V. Buonassisi, G. Sato, A. I. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **48**, 1184 (1962).
37. W. B. Pratt and L. Arranow, *J. Biol. Chem.* **241**, 5233 (1966).
38. E. V. Jensen, M. Numata, P. I. Brecher, E. R. DeSombre, *Biochem. Soc. Symp.* **32**, 133 (1971).
39. K. Fuxe *et al.*, *Endocrinology* **117**, 1803 (1985).
40. M. G. Govindan, *Exp. Cell Res.* **127**, 293 (1980).
41. A. C. Towle and P. Y. Sze, *J. Steroid Biochem.* **18**, 135 (1983).
42. B. Gametchu, unpublished observations.
43. M. Huet-Minkowiski, J. C. Gasson, S. Bourgeois, in *Drug and Hormone Resistance in Neoplasia: Basic Concepts*, N. Bruchovsky and J. H. Goldine, Eds. (CRC Press, Boca Raton, FL, 1982), vol. 1, pp. 79–94.
44. J. Stevens, Y-W. Stevens, H. Haubenstock, in *Biochemical Action of Hormones*, G. Litwack, Ed. (Academic Press, New York, 1983), vol. 10, pp. 383–446.
45. J. C. Gasson and S. Bourgeois, *J. Cell Biol.* **96**, 409 (1983).
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# Technical Comments

## Solving Linear Equations

For some time I have been disturbed by the reporting of applied mathematics in *Science*. However, the article "Solving linear systems faster" by Gina Kolata (*1*) contains so many misleading statements about a field I know well that I feel obliged to respond.

The thesis of the article is contained in its heading: "A new method [developed by Victor Pan and John Reif] that exploits parallel computations promises to have a real impact on practical problems." At the very least this statement is a gross exaggeration, and to support it the article presents a distorted view of algorithms and computing practices in the real world, not to mention of Pan and Reif's work. To give my comments focus, I will begin with a summary of the article.

1) The computer solution of linear systems is a problem with important applications in many areas—weather prediction for one. There are two classes of methods for solving such systems: direct and iterative. Of the former, the most commonly used method, called Gaussian elimination, has the drawback that it is inherently sequential and therefore not amenable to parallel implementation. Moreover, Gaussian elimination is unstable in the sense that rounding error can cause it to produce inaccurate answers. Consequently, most people use an iterative method to improve the solution obtained by Gaussian elimination.

2) Iterative methods are "stable, efficient, and amenable to parallel processing." They start with an approximate inverse of the matrix of the system and converge to the true inverse, after which the system can be solved by multiplying the right-hand side of the system of the inverse. The chief drawback to these methods is that they require an approximate inverse to start the iteration.

3) The first such iterative method was published by Schultz in 1933 and has been rediscovered several times since—by Pan and Reif among others. However, Pan and Reif also discovered how to obtain a starting approximation, thus "solving a problem that had been stumping researchers for 50 years." Pan and Reif also observed that by using about $n^3$ processors (which in theory can be reduced to about $n^{2.5}$) they could parallelize the algorithm so that it would be faster than previously proposed stable algorithms. Moreover, their method can be made more efficient for the very important class of sparse systems, in which most of the coefficients are zero. Although current parallel computers have too few processors to realize the full potential of the algorithm, bigger ones are in the offing, and according to Ronald Rivest of the Massachusetts Institute of Technology "the possibilities look very exciting."

This, I believe, is a fair summary of what is in the article. I have stuck to the words and quotations in the article itself and tried to avoid interpolating material from Pan and Reif's paper.

To put the matter of applications in perspective, it should be noted that the equations of weather forecasting, like those of many other applications, are not linear algebraic equations but nonlinear partial differential equations. Although the solution of linear systems sometimes plays an important role in solving such equations, it is only part of the total computation. For this reason speedups in the solution of linear systems often give disappointingly small speedups in applications.

The statement that Gaussian elimination is inherently sequential is incorrect. Indeed Pan and Reif themselves point out that by the use of $O(n^2)$ processors Gaussian elimination can be reduced from an $O(n^3)$ algorithm to an $O(n)$ algorithm. Perhaps more important, given the small size of current parallel processors, is the fact that with only $n$ processors Gaussian elimination can run in $O(n^2)$ time. All of this is well known to researchers in parallel computations.

The question of the stability of Gaussian elimination requires some background. Numerical analysis grade linear systems according to their difficulty of solution. At one end of this continuum are well-conditioned problems whose solutions are insensitive to small perturbations in the coefficients; as one moves away from this end one encounters problems that are increasingly sensitive, the so-called ill-conditioned problems. No method can be expected to solve ill-conditioned problems to a given precision without using higher precision somewhere in the computations. Gaussian elimination does about as well as an algorithm can be expected to do. It solves well-conditioned problems accurately, and for all problems, whatever their condition, it produces a solution that almost exactly satisfies the equations (although it may be very inaccurate). This means that if Gaussian elimination fails to give an accurate solution, there is something wrong with the equations, which must be reexamined. For this reason, among others, people with real applications rarely use iterative methods to refine the solutions computed by Gaussian elimination.

There are many types of iterative methods, not one, as the article seems to say. Of these many types, the article confounds two: methods of iterative refinement, which are occasionally used to touch up solutions computed by direct methods, and iterative methods for computing inverses. Moreover, it omits the most widely used class of methods: those that work directly with the system and do not require approximate inverses (although the inverse of an approximation can be used to speed some of them up). All these methods vary in efficiency and potential for parallelization. None of them will