proportion of adherent and nonadherent cells was approximately the same in both groups. Adherent cells had a slightly higher proportion of CFC in both groups as compared with unseparated and nonadherent cells. In the CML group the number of CFC was considerably reduced due to the effective treatment regimen ($P < 0.003$) (Tables 1 and 2).

Table 3 shows the distribution of *BCR/ABL*-positive CFC among the fractions as tested by FISH. A reduction of *BCR/ABL*-positive CFC was not found in any of the fractions. The relatively low number of colonies analyzed is the reflection of the situation that patients treated with HU and/or IFN have low leukocyte counts and that their blood contains only few CFC.

Distribution of BCR/ABL-positive clonogenic cells in plastic-adherent and nonadherent PBMNC fractions from CML patients at diagnosis or after therapy failing induction of hematologic response

CML blood from patients at diagnosis was characterized by high leukocyte counts. Corresponding high numbers of CFC could be found per ml blood or 10^6 MNC, respectively. However, the proportion of adherent and nonadherent cells was comparable to that of PBMNC from CML patients in stable chronic phase. In contrast, the concentration of CFC in the adherent MNC fraction from blood of patients at diagnosis was low (Table 4), even relatively lower than in the adherent CFC of BM cells from healthy control persons ($P < 0.04$) (Table 5). Obviously, adherent CFC of normal BM and CML blood represent a certain subpopulation of hematopoietic cells characterized by the same surface charge.

We have so far FISH-analyzed colonies from 17 CML patients at diagnosis (data not shown here). Only three of them had some *BCR/ABL*-negative colonies. The other ones possessed 100% positive colonies so that to perform FISH tests on cell fractions seemed to us unnecessary. This was also supported by the observation that CML patients in acceleration or blast crisis, who showed almost exclusively *BCR/ABL*-positive CFC in the blood, had so in the adherent and nonadherent fractions (Table 6). The proportion of adherent and nonadherent PBMNC from the same patients resembled that of normal PBMNC (Tables 1 and 7).

Discussion

Adherence of cells to plastic materials depends on electrostatic interactions of the plastic surface and the more or less negatively charged cell membrane. The membrane potential is not static but is influenced by several factors, particularly by changes of the membrane

Table 1. Proportion of plastic-adherent and nonadherent PBMNC from healthy control persons. Colony formation by separated cell populations.*

Control persons	MNC x 10^4 isolated/ml blood	Adherent cells x $10^4/10^6$ MNC = %	Nonadherent cells x $10^4/10^6$ MNC = %	Loss by cell death	No. of colonies/10^6 unseparated MNC	No. of colonies/10^6 adherent MNC	No. of colonies/10^6 nonadherent MNC
1	2.05	1.11	31.94	67.0	54	0	33
	1.93	0.83	37.50	61.7	42	78	50
2	2.23	2.17	28.08	69.7	118	733	33
3	1.59	9.08	21.50	69.4	236	292	122
	2.22	2.82	28.67	68.5	217	102	133
4	2.23	4.09	33.33	62.6	155	383	41
5	1.94	3.28	17.75	78.0	323	1055	122
	3.13	1.98	30.25	67.7	132	318	116
6	1.53	2.43	24.33	73.3	102	181	56
7	0.93	10.16	48.48	41.3	369	353	115
	1.72	5.32	32.33	26.4	156	308	88
8	2.11	2.71	40.67	56.6	129	461	34
9	1.39	3.23	44.83	52.0	164	422	83
10	1.68	2.19	32.83	65.0	231	351	73
11	3.85	1.57	45.83	52.6	57	113	27
mean	2.04	3.53	33.22	60.8	166	343	75
median	1.94	2.71	32.33	65.0	155	318	73
minimum	0.93	0.83	17.75	26.4	42	0	27
maximum	3.85	10.16	48.48	78.0	369	1055	133

*Comparison of the data by the Student's t-test showed a significant difference with the data presented in Table 2, but not with those presented in Table 7.

Table 2. Proportion of plastic-adherent and nonadherent PBMNC from CML patients in stable chronic phase. Colony formation by separated cell populations.*

Patient No.	MNC x 10^6/isolated/ml blood	Adherent cells x $10^4/10^6$ MNC = %	Nonadherent cells x $10^4/10^6$ MNC = %	Loss by cell death	No. of colonies/10^6 unseparated MNC	No. of colonies/10^6 adherent MNC	No. of colonies/10^6 nonadherent MNC
1	2.49	4.23	21.77	74.0	6	32	13
2	1.31	3.86	90.90	5.1	25	31	14
	1.95	7.73	19.12	73.2	28	38	16
3	0.62	2.74	60.00	37.3	3	25	10
	0.95	2.00	73.60	24.4	8	28	10
	0.91	1.00	38.80	60.2	0	0	2
	0.88	2.80	30.60	66.6	no colonies	no colonies	no colonies
4	1.66	7.60	70.75	21.8	33	34	59
	2.54	6.90	24.70	68.4	27	22	no data
	2.28	15.22	79.17	5.6	11	21	14
5	1.73	6.06	40.00	53.9	11	18	3
6	1.90	7.60	58.70	33.7	110	275	156
	1.47	8.77	37.94	53.3	65	42	15
	1.18	6.25	53.55	40.4	117	77	37
	1.39	8.33	53.44	38.3	4	10	1
	1.17	3.06	44.44	52.5	80	101	11
7	2.36	2.19	33.83	64.0	116	254	128
8	3.34	7.92	48.61	43.5	1	3	1
9	2.35	11.25	50.72	38.1	7	1	1
	5.51	8.96	36.46	54.5	3	1	2
mean	1.90	6.22	48.34	45.4	34	53	27
median	1.70	6.58	46.53	48.0	11	28	12
minimum	0.62	1.00	19.12	5.1	0	0	1
maximum	5.51	15.22	90.90	74.0	117	275	156

*Comparison of the data by the Student's t-test showed a significant difference with the data presented in Table 1.

permeability, which alter the electrochemical gradient of ions across the plasma membrane. Receptor-ligand binding is a physiological factor that may change the membrane potential.

Assuming that cells expressing the same pattern of surface molecules belong to the same lineage and stage of differentiation, they would have an identical membrane charge and thus the same capacity to bind to the plastic surface of tissue culture flasks. However, the surface charge of cells may change during the cell cycle so that different cell types may contribute to the population of adherent cells.

Monocytes-macrophages preferably adhere to plastic materials. However, there are also several other types of cells among BM and PBMNC preparations that may bind selectively. Their binding capacity varies, and therefore constant shearing forces have to be applied for separating comparably adherent and nonadherent cell fractions.

We have used normal and CML PBMNC for adhesion to the plastic surface of tissue culture flasks. The selected cell type to be investigated was the CFC, thus having constricted our test system to functionally identical hematopoietic precursor cells.

Similar experiments have been performed by Grand et al. (1997), who studied magnetic activated cell sorting (MACS) column-isolated $CD34^+$ cells from CML blood for adherence separation. As detected by FISH, they found that the concentration of Ph^- cells in the adherent $CD34^+$ cell fraction was three-fold higher than in the nonadherent fraction, although both adherent and nonadherent fractions contained Ph^+ and Ph^- cells.

As we show here, a preferential adherence of *BCR/ABL*-negative CFC from CML PBMNC could not be obtained. There was even a slightly higher proportion of *BCR/ABL*-positive colonies in the adherent as compared with unseparated or nonadherent fractions.

FISH analyses of colonies allow an exact enumeration of *BCR/ABL*-positive and negative CFC in a cell population. When combined with Wright-Giemsa staining, the colonies can be assigned to the lineage commitment of